411

INHERITANCE IN NICOTIANA TABACUM

I

A REPORT ON THE RESULTS OF CROSSING CERTAIN VARIETIES

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THOMAS HARPER GOODSPEED
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CONTENTS

I. INTRODUCTORY

The inception of the work on the various species of *Nicotiana* grown and bred in the University of California Botanical Garden has already been sketched in a previous number of this series (cf. Setchell, 1912). As stated there, the original intention was to assemble a collection of tobacco plants simply as a portion of the outfit of the Botanical Garden for general instruction and display. So great was the variety and evident misapplication of the names under which the seeds were received, however, that it seemed advisable to attempt to determine, as definitely as possible, the status of each plant.

In this connection, the work of Comes, in particular, came under consideration and especially his views as to the origin and interrelationships of the various cultivated forms belonging to the *Tabacum* group. Comes (1899, p. 4 and elsewhere) regards the numerous cultivated forms of tobacco as having originated in various ways from certain fundamental varieties. He estimated that there are six of these fundamental varieties of *Tabacum*, and he supposed the large number of various and seemingly more or less intergrading forms to have arisen through the influence of the forces of acclimatization, adaptation, hybridization, and selection. Of these, undoubtedly, the greater variations have been produced and perpetuated, according to the ideas of Comes, through hybridization and selection. In his monograph (1899) and in his later more exhaustive treatise (1905), Comes has attempted to estimate just which of his six fundamental varieties of *Tabacum* have coöperated in producing each one of the cultivated ''races'' so far as known to him.

The statements of Comes as regards the constitution of his various races seem to have been based on the results of morphological study rather than upon breeding analysis. The advisability occurred to the senior author of attempting to test Comes' hypothesis by selecting varieties seemingly fundamental in type, and through hybridization

and selection attempting to secure constant races exhibiting various recombinations of the parental characters. The work thus conceived has been carried out in detail in a certain few but seemingly characteristic cases. Several different crosses were made in 1909, the first filial generations were grown in 1910, and each year since that time has seen successive filial generations in the field.

Although the *Nicotiana* investigations were originally designed to attack experimentally a comparatively simple and definite problem, they have since been greatly amplified in scope. At the present time three rather distinct lines of investigation are actively in progress, viz.,

1. Mendelian inheritance in *N. Tabacum.*
2. Inheritance of quantitative characters.
3. Studies of interspecific hybrids.

The recent appearance of bud variations in hybrid lines favorable for an analytic study of that phenomenon has resulted in the addition of another research project. Now it has been found that, although seemingly distinct, progress in these separate lines of investigation is more or less interdependent. In particular it has been found that certain of the phenomena exhibited in interspecific hybrid populations from crosses between *N. sylvestris* and varieties of *N. Tabacum* require for satisfactory analysis and explanation an accurate and detailed knowledge of the Mendelian differences which exist among the particular varieties of *N. Tabacum* that have been used in those investigations. Accordingly in later years these studies of hybrids between varieties of *N. Tabacum,* originally designed merely to test experimentally the interrelationships existing among such varieties, have been directed toward a specific Mendelian analysis of the germinal differences existing in a selected set of varieties.

With this change in emphasis has come a full appreciation of the difficulties of Mendelian studies in *N. Tabacum.* It has been very evident that, for the most part, the character differences among varieties of *N. Tabacum* do not rest upon a simple genetic basis; on the contrary, they often depend upon very complex and involved Mendelian differences; so that in segregating populations it is often impossible to demonstrate the existence of definite, discontinuous character classes. Not uncommonly the members of such populations may be arranged in series connecting by imperceptible differences the most extreme character expressions in the population.

But although complex intergrading segregation has often been observed in F_2, it has not been found that such complex segregation

persists in subsequent generations in the hybrid lines. On the contrary, it has been found, as will become evident in a study of the experimental material communicated herewith, that a great simplification occurs in the segregation in F_3 and subsequent generations, and that continuous segregation gives way to discontinuous just as might be expected from Mendelian theory. By observing the segregation in the consecutive generations of hybrid lines which have become homozygous in most of their loci through self-fertilization, it is possible to obtain some idea of the Mendelian factor pairs involved in the character contrasts and of their relations to one another. It has also proved possible by a few years of self-fertilization to establish stable lines representing recombinations of parental characters. By investigating the interrelations among such stable derivative lines, which obviously should differ in fewer factors from one another and from the original parental varieties than the parental varieties differ from each other, it would seem possible to develop an indirect mode of attack by which the Mendelian analysis could be refined to any desired extent. The original plan of the investigation, therefore, having as its purpose a demonstration of the possibility of securing by hybridization stable derivative lines representing recombinatons of characters contained in the parents and comparable to the numerous existing varieties of *N. Tabacum*, has been diverted into a detailed study of Mendelian differences among a typical set of *N. Tabacum* varieties.

II. PLAN OF THE WORK

In the introductory paper the senior author has discussed the fundamental types of *N. Tabacum*, and as indicated there, has expressed a preference for selecting some five fundamental varieties, or species, as representing the basal morphological elements found, or seemingly to be detected, in cultivated races of *N. Tabacum*. There is no necessity for discussing further, at present, the reasons for preferring the particular types selected by us as against those of either Comes or Anastasia (1906), since the fundamental conceptions agree sufficiently well and the important thing has been to make a beginning in experimentation by using varieties which present seemingly fundamentally different character complexes in most characteristic form in plants breeding true to type in the pure line. Certain reasons for selecting a particular type or types will be discussed in connection with the

consideration of the various crosses. Besides the ''fundamental'' types, there have been selected for crossing certain other types, possibly fundamental, or in some cases derivative, which have been employed for testing the inheritance of some particular character or group of characters. All of these have been described in the first paper of this volume.

The taxonomic problems in *N. Tabacum* do not appear to differ from those presented by many other species of cultivated plants. Barley, maize, oats, rice, wheat, among others, exhibit a similar diversity of forms with more or less obvious class distinctions. In these as in *N. Tabacum* it appears to be an easy task to shuffle and recombine characters indefinitely. Clearly there can be no segregation of forms into distinct species on genetic grounds; the basis of speciation, if any, must depend either upon convenience merely or what amounts to practically the same thing, upon elevation of certain Mendelian character contrasts to a higher rank in classification than others. Since the taxonomic problem, therefore, is not strictly a genetic one, it seems best to follow general usage in this respect, referring all the polymorphic assemblage of forms to the one species *N. Tabacum,* and regarding the several races included thereunder as varieties of equal rank.

The varieties employed in this series of investigations are: *N. Tabacum* var. *alba*, U. C. B. G. 30/06, previously described by Setchell as ''White'' Tobacco; *N. Tabacum* var. *angustifolia*, U. C. B. G. 68/07, previously described by Setchell as *N. angustifolia; N. Tabacum* var. *calycina*, U. C. B. G. 110/05; *N. Tabacum* var. *macrophylla*, U. C. B. G. 22/07; and *N. Tabacum* var. *virginica*, U. C. B. G. 78/05, previously described by Setchell as *N. Tabacum* ''Maryland.'' In each instance the University of California Botanical Garden (U. C. B. G.) number contains in the numerator the accession number of the year given in the denominator. The varieties have in the majority of cases been grown in pure lines from the date of their receipt. In order to avoid needlessly encumbering the text with scientific names, the varieties mentioned above will be referred to by their varietal designations only, and when reference is made to the whole group the species name *Tabacum* will be used alone.

Three series of cultures are described in the present article: the *angustifolia-macrophylla* series, which has been derived from reciprocal crosses of *angustifolia* and *macrophylla;* the *calycina-virginica* series, derived in the same way from *calycina* and *virginica;* and the *alba-*

macrophylla series, from *alba* and *macrophylla*. In the course of the investigations other crosses were made between different varieties of *Tabacum* and to a limited extent between other species of *Nicotiana;* but the principal attention has been paid to the three crosses noted above, and they and their progenies alone will be considered in the present paper. It may be said at this point that the different varieties of *Tabacum* cross readily with one another, giving an abundance of good viable seed. The hybrids are uniformly self-fertile.

The methods of hybridization used need not be considered here, because they have been described in detail by Goodspeed (1912) elsewhere in this series. The particular refinements of technique which must be employed in sowing the seed, on account of its very small size, have also been there described. It might be well to state, however, that the most refined methods doubtless will not prevent the occasional appearance of a stray plant in the cultures. The danger of contamination arises not only during the sowing of the seed, but also when the bags are placed over the unopened buds. It is very easy to include a few stray seeds under the bag, for their small size makes it almost impossible to detect them in the coarse, sticky indumentum of the plant. In spite of these obvious difficulties, however, the number of plants that have certainly been strays has been very small. Their rare occurrence indicates clearly that the technique employed has been very successful.

III. ANGUSTIFOLIA–MACROPHYLLA SERIES

This series has received the most attention since the parents are so distinctly different, and the results have consequently been more complex than those which have followed the crossing of any other pair of *Tabacum* varieties. As will be demonstrated below, F_2 seemed at first hopeless in its variety of segregation. Later generations, however, exhibited so much less, or so little variety in their segregation products that it was easy to obtain new permanent combinations of characters or "fixations." Certain of its segregants have been followed out to F_7, and have also been crossed back on the parents which they most closely resembled.

Six successful crosses were made. Of these H_2 and H_3 had *macrophylla* for the male and *angustifolia* for the female parent, while H_4, H_5, H_{15}, and H_{16} were reciprocal crosses. As a matter of convenience

the generations later than F_1 were grown only from H_2 and H_4, the larger number from H_4. The predominance of H_4 in the later families selected for the continuation of the work was not, however, due to any especially different behavior evidenced in that particular series.

1. PARENTS OF THE ANGUSTIFOLIA–MACROPHYLLA SERIES

By selecting *angustifolia* and *macrophylla* for crossing, two varieties were obtained which resemble each other in height and general habit, but which differ strikingly in leaf and flower characters. The differences are sufficiently great to lead one to regard them as belonging to different species; in fact, all five *Tabacum* varieties selected by us as possibly fundamental differ sufficiently among themselves to be regarded as species in the *Tabacum* section rather than as varieties. It is not our intention, however, to emphasize this point, since any discussion would of necessity lead to a general survey of all the known varieties and races at present included under *Tabacum*. If, however, these five, viz., *angustifolia, macrophylla,* "*Cavala,*" *Maryland,* and "*Brazilian*" (cf. Setchell, *loc. cit.*) could be considered by themselves as wild plants, it seems to us that any taxonomist of the present day would certainly award to each of them the rank of a separate species. These considerations should be borne in mind in estimating the significance of the results obtained through crossing.

Angustifolia, U. C. B. G. 68/07, is a variety which has long been known and which is represented in our breeding experiments by a pure line very closely approximating the type. It has been figured and discussed by one of us (Setchell, *loc. cit.,* p. 9, pl. 7). The photograph given there is of a young plant just coming into flower and consequently does not represent the habit of the plant in full blossom or in fruit, after the full number of laterals is developed. A plant in the height of its vigor is represented in plate 55, figure 1. In stature *angustifolia* belongs to the low corymbose group of *Tabacum* varieties, which also includes the forms bred in the University of California Botanical Garden under the names *calycina* and *macrophylla,* and which is in decided contrast to the tall, more "racemose" (although these may be "corymbose" at the top) forms such as *alba* and *virginica.*

In height *angustifolia* varies from 75 to 120 cm. The central axis develops its corymbose panicle of short racemes first, but it is usually soon overtopped by the successive laterals developed basipetally, each

lateral, in turn, developing a corymbose cluster of racemes, rising more or less above its predecessors. The result is that the whole plant has the short corymbose habit mentioned above. The stems and branches of *angustifolia* are comparatively slender, being much more slender than those of *macrophylla*, or those of any other of the *Tabacum* varieties except those of *calycina*, which are very similar.

The leaves of *angustifolia* are alternate and distinctly and moderately long petiolate. The blade of the lower leaves is ovate-lanceolate, tapering above to a long, curved point, more or less conduplicate below and with the rounded bases unequal. Above, the leaves are less conduplicate, more so even at the base, with the petiole shorter, while the uppermost (bracts) become almost sessile and narrowly lanceolate even to almost linear in outline. The normal petiole is naked at the base and in the middle portion, but the base of the blade is slightly and narrowly decurrent along the upper portion. Occasionally a petiole shows a narrow wing throughout its length and at times the petioles of all the leaves on certain plants are more or less winged, but the majority of the plants have naked petioles (cf. also Goodspeed and Clausen, 1917, p. 306, pl. 46, right-hand figure). The leaves of *angustifolia* have also a very characteristic drooping habit, much more pronounced than in any other *Tabacum* variety except *calycina*. In older plants, after capsule formation has well advanced, all the leaves are hanging obliquely downwards.

The flower of *angustifolia* is distinctive and differs in details of shape and color from that of any other *Tabacum* variety, and especially from that of *macrophylla*. The general shape of the flower is that of all the *Tabacum* section, but the corolla is much more slender and more gradually infundibuliform than that of any of the other varieties reported here. The calyx is broadly campanulate, prolonged above into 5 long, but unequal, linear-lanceolate, pointed lobes, of which one is longer than the remaining four and gives the calyx a zygomorphic appearance. The corolla is narrow and tubular below the middle, expanding rather gradually and evenly above into a conical infundibulum which bears the spreading, deeply 5-lobed limb at its summit. The length of the tube of the corolla is about 6 cm. and its greatest diameter about 7 mm. The limb of the corolla, at first erect (opening bud), then horizontal, finally becomes somewhat deflexed and measures about 3 or 3.5 cm. across. It is divided almost to the tube into 5 lobes which are ovate-lanceolate with long, narrow, tapering tips. The lobes of *angustifolia* are much longer and have narrower tapering tips than

those of any other *Tabacum* variety, and in this respect are in direct contrast to those of *macrophylla*. The lobes are unequal and give a slight suggestion of zygomorphism to the corolla. The stamens are inserted on the lower portion of the tube and are usually slightly exserted in anthesis. The pistil possesses the usual 2-celled ovary, long, slender style, and thick, slightly bilobed stigma, more or less exserted in late anthesis, characteristic of the genus *Nicotiana*. The color of the corolla is a light, through lively, pink, much lighter than the red of *macrophylla*. The capsule at maturity is slightly flattened longitudinally, is broadly lanceolate in profile, tapers above into an acuminate apex, and is about 25 mm. high and 8 to 9 mm. thick. It is the most slender of all the capsules borne by the various *Tabacum* varieties and in decided contrast to the stout capsule of *macrophylla*.

In plate 55, figure 1, is illustrated a plant of *angustifolia* at the height of its blooming period. Typical features of the plant are shown in the line drawings of plate 56. Photographs of typical leaves are shown in plate 58, where they may be compared with photographs of the leaves of *macrophylla*. Photographs of the flowers are reproduced in plate 60, where they may be compared with those of *macrophylla* and of the hybrids between these two varieties.

Macrophylla, U. C. B. G. 22/07, has already been discussed and figured by one of us (cf. Setchell, *loc. cit.*). The original seed was obtained from Comes, but the plants do not correspond to his figures (cf. Comes, 1899, pl. VIII) either as to habit or shape of leaf. They differ also from his description in these same respects. The flower, however, agrees, and it seems best to retain for it the name under which we have cultivated it.

The habit and height of *macrophylla* are both very similar to those of *angustifolia*. The habit is low corymbose, the central axis bearing a panicle of corymbose racemes and the laterals arising one after the other bearing similar inflorescences and equaling or overtopping the central axis. The stems and branches are stouter than those of *angustifolia,* however, and this, together with the broader, more solid looking leaves which do not droop so much as those of *angustifolia,* give a mature plant of *macrophylla* a much more robust appearance than is the case with a mature plant of *angustifolia*. The plant figured in the first number of this volume (pl. 6) was young. An older plant shown herewith on plate 55, figure 2, is in full blossom and beginning to ripen its capsules, and gives a better idea of the habit of a well grown plant.

The leaves of *macrophylla* are sessile by a partially clasping base and possess two basal lobes partially clasping the stem. The general shape is obovate, the widest portion being above the middle. The leaves taper gradually to the broad clasping base below and abruptly to a narrow more or less acuminate tip above. The surfaces show the secondary veins branching at a more obtuse angle than do those of the leaves of *angustifolia*. The color of the leaves is a dark green in *macrophylla* and more of a yellowish green in *angustifolia*. In every way, then, the leaves of the two parents differ from each other as much, in fact, as do the leaves of many species.

The flowers of *macrophylla,* while of the same general type as those of *angustifolia,* differ in details of shape and color. The flowers of *macrophylla* are about 4 cm. long. The calyx is broadly ovate in profile, deeply cut into 5 broad and somewhat unequal lobes. The corolla tube is stout cylindrical (about 5 mm. in diameter) below, broadening suddenly into a stout infundibulum above (about 10 mm. in diameter). The limb is at right angles to the tube, is about 23 mm. across, and is more or less pentagonal with 5 shallow sinuses. The color of the corolla is deep red fading to an almost lilac tint after anthesis. On the limb are 5 triangular lighter areas, one having the narrow apex at each sinus and the broad base at the top of the tube. In the much darker color, in the broader tube and stouter infundibulum, and in the barely appreciable lobing of the limb, the corolla of *macrophylla* is the antithesis of that of *angustifolia*. In stamens and pistil, the flower of *macrophylla* shows little variation from that of *angustifolia*.

The capsule of *macrophylla* is broadly ovate, tapering abruptly to a mucronate tip. It is about 2 cm. high and about 1.5 cm. in diameter, contrasting very decidedly with the comparatively slender capsule of *angustifolia*.

A typical plant of *macrophylla* is shown in plate 55, figure 2. Typical features of the plant are shown in line drawings in plate 57. Photographs of leaves are reproduced in plate 58, where they may be compared directly with those of *angustifolia*. In plate 60 its flowers may be compared directly with those of *angustifolia* and with those of the hybrids.

It has seemed best to call attention to the characters of and differences between these two varieties, parents in the first set of crosses to be discussed, in order that the behavior of their hybrid progeny may be clear. In height and habit there is a close agreement, but in leaf, flower, and fruit there are sufficient differences to mark them as separate species.

2. F_1 OF THE ANGUSTIFOLIA–MACROPHYLLA SERIES

In late July of 1909, some 7 crosses were made between *angusti-folia* and *macrophylla,* 6 of which, as stated above, were successful. H_1, H_2, and H_3, as they were designated, involved *angustifolia* as the female plant, while H_4, H_5, H_{15}, and H_{16} were reciprocals. No seed was obtained from H_1, but all the other 6 crosses gave a fair yield. The usual care (cf. Goodspeed, *loc. cit.*, pp. 129–131) was taken in cleaning and sowing the seed. This was done in the spring of 1910, germination was good in all cases, and 337 plants, distributed as follows, came to maturity and seed bearing. The family of H_2 had 56, H_3 had 60, H_4 had 47, H_5 had 58, H_{15} had 55, and H_{16} had 61 plants.

A survey of all these plants showed in general a remarkable uniformity in habit. A certain amount of difference was to be detected on careful scrutiny, but little if any greater than that which is exhibited among a large number of individuals of one or the other parent. In height, F_1 showed exactly the same variation as the parent, the central axes varying from 65 to 145 cm., but largely varying from 90 to 120 cm., while the laterals rose to 150 cm. Some rows showed uniformly higher, others uniformly lower plants, the differences probably being due to different soil and water conditions. The habit (see pl. 61) was low corymbose and the general appearance as to stoutness seemed more or less intermediate between the two parents. The leaves in shape, size, etc., very closely resembled those of *angustifolia.* There was some appreciable variation in the leaves, however, and often considerable variation on the same plant, a characteristic of *angustifolia* which has already been mentioned. Plate 59 reproduces photographs of different types of leaves obtained from F_1 plants. The blade is broadly elliptical ovate with the lateral veins at an obtuse angle, much as in *macrophylla.* The base is rounded, or even slightly cordate in some leaves, while the tip is more blunt. These characters seem, at least, to indicate an influence of *macrophylla.* The leaf, however, is distinctly petiolate, but the petiole is not so long as in *angustifolia.*

The petiole is definitely winged and the wings are expanded at the base into auricles, which are often triangularly decurrent along the internode of the stem. This wing is usually present in all the plants of F_1, but some leaf or leaves on a plant may lack it, and in some plants it is only slightly developed, or at least, is without auricles. The wing was from 5 to 7 mm. wide on some leaves.

The leaf of $10F_1H_{15}P_7$ represented in plate 62 even more closely resembles the typical leaf of *angustifolia.* The wing along the mar-

gins (or edges) of the petiole is narrow and is prolonged as a slight ridge to the internode and is decurrent (?) or can be traced as it bends sharply downwards. Such wings are, at times, found in pure bred *angustifolia* plants. This leaf, then, resembles the *angustifolia* leaf fairly closely, but differs from its ordinary expression in the more tapering base, in being less distinctly conduplicate, in tapering more abruptly toward the tip, in having shorter petioles and in having more of a wing on the margins of the petiole.

The flower of F_1 (see pl. 60) resembles that of *angustifolia* more than that of *macrophylla*. The color is deep pink, decidedly of a deeper shade than is the flower of *angustifolia*, yet far from the red of *macrophylla* and in a way intermediate between the two. There is no trace, on the limb of the corolla of F_1, of the 5 white triangle-shaped areas so characteristic of the limb of the corolla of *macrophylla*. The infundibulum, while possibly slightly stouter than that of the flower of *angustifolia*, is not so stout as that of the flower of *macrophylla*. In length the flower of F_1 averages about 4 to 4.5 cm. as against an average of 6 cm. in *angustifolia*, and of 4 cm. in *macrophylla*. The tube averages about 3.5 to 5 mm. in diameter below, as contrasted with 2.5 to 3 mm. as an average in *angustifolia* and 5 mm. in *macrophylla*. The infundibulum in the corolla of F_1, while neither abrupt nor so stout as that of the flower of *macrophylla*, is noticeably more abruptly enlarged and stouter than that of *angustifolia*. The limb of the corolla in F_1 averages 2.5 to 3.25 cm. in greatest diameter, while that of *angustifolia* averages 3 to 3.5 cm. and that of *macrophylla* averages about 2.3 cm. in greatest diameter. The lobes of the corolla in F_1 are about half the width of the limb from tube margin to extreme tip of lobe, while in *angustifolia* they are about two-thirds of this and in *macrophylla* they are only one-third or even less. The lobes, also, are decidedly broad at the base, particularly so as compared with their length. In general, then, the corolla of F_1, while closer to that of *angustifolia*, shows by its stouter tube, more abrupt and more swollen infundibulum, intermediate spread of limb, less deep lobing, shorter and broader lobes, and deeper shade of pink, definite influences of *macrophylla* also.

The capsule of F_1 is broader than that of *angustifolia*, but narrower than that of *macrophylla*. There is greater variability in horizontal diameter in the capsule of F_1. The flower and fruit of F_1, then, although no careful biometric study has been made, are intermediate between those of the two parents, yet incline more toward *angustifolia* than toward *macrophylla*.

In general, then, a survey of F_1 shows throughout a series of ten
different families a uniformity of individuals as great as that exhib-
ited in either of the parents. Some few slight differences exist among
individuals both of F_1 and of the parents which may possibly be re-
ferred to lack of a completely homozygous conditon in the parents.
In characters in which the two parents differ, whether in color of
flower, quantitative corolla character complexes, capsule character com-
plexes, or leaf character complexes, the F_1 hybrid exhibited through-
out a character expression intermediate between that of the two
parents.

3. F_2 OF THE ANGUSTIFOLIA–MACROPHYLLA SERIES

In 1911, there were selected as parents for the F_2 13 plants from
H_2 and 12 from H_4. Twenty-one families of approximately 50 plants
each were set out in the field. On account of the great diversity shown
in these populations, it was found impossible to study individually
each of the thousand plants grown; consequently particular attention
was paid to only 5 families from each hybrid. The other families were
gone over carefully, but nothing notably different was found in their
behavior. All fifty plants survived in each family except in the last,
viz., $11F_2H_4P_{43}$, where only 48 came to maturity. There were then
498 plants of F_2 under more careful observation, representing both
the cross and its reciprocal, with about 550 remaining for only casual
examination.

As might have been expected, there was a great variety of plants
resulting and segregation as to differences in combination of characters
of flower, fruit, and leaf was little short of bewildering. An attempt
was made to study and arrange these combinations, but it was found
to be impossible. A careful survey, however, was made of the popu-
lations and a tabulation of characters was attempted. Some 16 fairly
readily separable types, based on leaf characters, were distinguished,
but between these closely approaching types others were to be found
of intermediate and overlapping character. One each of the types
selected was drawn, and these drawings are reproduced in plates 63
to 78.

A glance at these plates, which were carefully drawn to scale, will
show something of the nature of the combinations of characters of the
two original parents. Type 1 (pl. 63) shows a close approximation,
yet not an absolute reproduction, of *angustifolia*, while type 16 (pl. 78)
in a similar way is a close approximation to *macrophylla*. The other

14 types (pls. 64 to 77) are clearly intermediates approaching one parent more than the other, but types 12, 13, and 14 (pls. 74 to 77, inclusive) are decidedly different from either as to leaf, at least, and type 10 (pl. 72) is of another altogether different form, although all of these leaf shapes are connected to a greater or less extent into one series of more or less gently intergrading forms.

As to the shape and dimensions of the corolla there is to be found a similar series of intergrading forms from the slender corolla tube with gradually expanding and slightly swollen infundibulum and deeply lobed limb of type 1 (pl. 63) to the corolla with stout tube, abruptly and considerably swollen infundibulum with slightly lobed limb of type 16 (pl. 78). In color the corollas vary from the light pink of *angustifolia* to the red of *macrophylla* and three shades are at times fairly readily distinguishable, the light pink of *angustifolia*, the deep pink of F_1, and the several nuances of the red of *macrophylla*.

The capsules also show various combinations from the slender gradually attenuated capsules of *angustifolia* to the stout, swollen, abruptly upwardly attenuated capsules of *macrophylla*. Both capsules and corollas approaching one parent may be found with leaves more closely approaching the other parent. In stature and habit the plants of all the 21 families were reasonably uniform and agreed in general in these respects with the parents and F_1, there certainly being no greater amplitude of variation in these respects than was to be found in the parental types.

Among the great variations, two characters seemed to stand out fairly clearly for rough statistical enumeration, viz., color of the corolla and the possession, or lack, of a petiole. Numerical data for these characters are given in table 1. Some care was taken to obtain a careful census of the families as regards each of the characters. As regards colors it was, as noted before, possible to distinguish three shades, or sets of shades, which were designated as light pink, pink, and red. In practice, however, it was usually difficult to distinguish the two shades of pink from each other. The red gave very little trouble.

In attempting to classify the plants of F_2 with respect to type of leaf base, more difficulty was experienced because of the variety of forms which were produced and the degree of intergradation which existed between forms. In judging the presence or absence of petiole, therefore, in these populations, the classification is faulty because of lack of knowledge of the genetic constitution of the various distinct forms and those which grade into them. In table 1 the plants are

thrown into the petiolate class if they were distinctly narrowed at the base, and whether naked or winged.

In F_2, then, there appears to be simple Mendelian inheritance in only one pair of the original character contrasts of the parents, namely, red versus pink corolla color. Here the hybrid is intermediate and F_2 segregates sharply into pink and red in the ratio 3 pink : 1 red. Within the pink class there is a more or less evident segregation into 2 pink : 1 light pink, but the shades intergrade so that no distinct line of demarcation exists between the classes. As respects leaf base characters, the segregation is so complex that no reasonable genetic analysis is possible. The numerical data for this latter character presented in table 1 are of value only in that they indicate a close agreement in segregation among F_2 families, thereby furnishing a rough statistical demonstration of the equivalence of the several families. The more definite data on leaf base characters are derived from generations subsequent to F_2.

TABLE 1

CLASSIFICATION OF F_2 PLANTS OF THE *ANGUSTIFOLIA-MACROPHYLLA* SERIES ACCORDING TO COROLLA COLOR AND LEAF BASE CHARACTERS.

Family Designations	Garden Numbers	Corolla Color			Leaf Base	
		red	pink	light pink	petiolate	non-petiolate
A	$11F_2H_2P_2$	12	23	14	42	7
B	$11F_2H_2P_3$	14	25	10	30	20
C	$11F_2H_2P_6$	13	21	15	34	14
D	$11F_2H_2P_7$	7	21	22	35	15
E	$11F_2H_2P_{13}$	15	20	14	30	20
F	$11F_2H_4P_2$	13	28	7	32	17
G	$11F_2H_4P_{35}$	12	29	8	35	14
H	$11F_2H_4P_{40}$	13	15	22	32	18
J	$11F_2H_4P_{41}$	8	31	10	31	17
K	$11F_2H_4P_{43}$	6	27	15	38	10
Totals		113	240	137	339	152

4. F_3 AND SUBSEQUENT GENERATIONS OF THE ANGUSTIFOLIA–MACROPHYLLA SERIES

From F_2 of H_2 and H_4, 20 plants were selected for further experimentation and families of 25 were determined upon as the unit. In all except four, the families of 25 each were successfully raised. Of one of the four only 14 plants were obtained, which were all that germinated, while in each of the other three families 24 plants were reared to maturity. Altogether, then, 486 plants were raised of the F_3 during the season of 1912. It was the intention to grow from each of the selected types, as drawn for illustration. Fifteen of the families from the type parents were successfully reared, but, in some way or other, the seed of type 4 ($10F_2H_2P_7P_{18}$) was not to be found, and, unfortunately, no note had been made as to whether or not any seed was produced. No complete sterility, however, was noticed in any members of F_2 of either H_2 or H_4, and the presumption is that F_2 seed of type 4 must have been lost in harvesting.

The variation within each family was decidedly less than that of the families of F_2. Of the 20 families reared wholly or in part, 4 families were very nearly uniform, varying in minor details only. Five families segregated only in corolla color, 4 segregated only in leaf base characters, and the remaining 7 segregated both in corolla color and leaf base characters. In table 2 are summarized the data as to gross behavior of these families of F_3. In those families grown in F_4 and subsequent generations, a definite attempt was made to fix the original characters of the F_2 type selection in a pure line. The genealogical relation of these selected lines to each other is shown in the chart reproduced herewith. The letters, A, B, C, etc., correspond to the F_2 family designations noted in table 1, and the numbers refer to type selection numbers corresponding to the type illustrations in plate 63 to 78, or, in the case of types 17 to 21, corresponding to those types as described in the succeeding accounts of the later generations.

TABLE 2

F_3 FAMILIES OF THE *ANGUSTIFOLIA-MACROPHYLLA* SERIES.

Type Nos.	Garden Numbers	No. of plants in families	Results in F_3
1	$11F_2H_2P_7P_{49}$	25	Segregated both as to leaf and flower color
2	$11F_2H_2P_3P_{30}$	25	Segregated both as to leaf and flower color
3	$11F_2H_2P_3P_{14}$	14	Segregated only as to leaf
5	$11F_2H_4P_{41}P_{14}$	24	Segregated both as to leaf and flower color
6	$11F_2H_4P_2P_{18}$	25	Uniform except as to length and development of wing of petiole
7	$11F_2H_2P_{13}P_{48}$	25	Uniform both as to leaf and flower color
8	$11F_2H_2P_3P_{41}$	25	Segregated as to leaf only
9	$11F_2H_4P_{41}P_8$	25.	Segregated both as to leaf and flower color
10	$11F_2H_4P_{41}P_{17}$	24	Segregated only as to flower color
11	$11F_2H_4P_{41}P_9$	25	Segregated only as to flower color
12	$11F_2H_4P_{41}P_{12}$	25	Segregated only as to flower color
13	$11F_2H_2P_3P_{44}$	25	Segregated only as to flower color
14	$11F_2H_2P_3P_{38}$	25	Segregated both as to leaf and flower color
15	$11F_2H_2P_3P_{10}$	25	Uniform, slight variation in tint and lobing of corolla
16	$11F_2H_2P_3P_8$	24	Segregated only as to leaf
17	$11F_2H_4P_{35}P_{27}$	25	Segregated only as to flower color
18	$11F_2H_4P_{35}P_{38}$	25	Segregated both as to leaf and flower color
19	$11F_2H_4P_{35}P_{43}$	25	Segregated both as to leaf and flower color
20	$11F_2H_4P_{40}P_{44}$	25	Uniform, close to *macrophylla*
21	$11F_2H_4P_{41}P_{29}$	25	Uniform, close to *angustifolia*

In F_2 primary selection for parents of subsequent generations was based upon the type of leaf borne by the plant, flower color being followed as a secondary matter. In order to systematize the discussion concerning F_3 and subsequent generations, six general types have been selected and named and the discussion of the families has been grouped
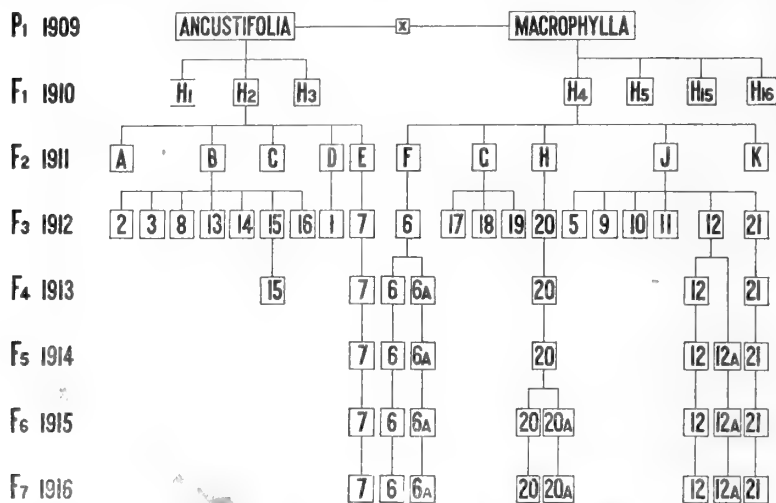
Fig. 1.—Chart showing the relationships of the various families of the *angustifolia-macrophylla* series. The different F_1 hybrids are connected with their female parents; no seed was secured from H_1. The F_2 family designations correspond to those given in table 1, and the numbers in later generations are the type numbers under which the populations are described in the text. F_4 and F_5 of type 12a were grown in 1914 and 1915 respectively.

under these headings. The six general types selected and the names given them are as follows:

a. STENOPHYLLA derivatives. As a series these approximate very closely in leaf shape to *angustifolia*. The distinguishing feature of this series is the possession of a distinct, long petiole.

b. LATIFOLIA derivatives, which are characterized by the possession of a broad leaf with a petiole shorter than that of *angustifolia*. The petiole in these types is more or less winged.

c. LANCEOLATA derivatives, which are characterized by the possession of a lanceolate leaf like that of type 13, illustrated in plate 75. This is a non-petiolate form.

d. LORIFOLIA derivatives, characterized by possession of long leaves with very narrow blades. The type specimen, type 12, is illustrated in plate 74. This also is a non-petiolate form resembling the LANCEOLATA derivatives, from which it differs in the extreme narrowing of the blade.

e. Auriculata derivatives. The typical form of leaf is that of type 10, illustrated in plate 72. The leaf blade of this form is characterized by an abrupt contraction of the blade at the base, nearly, if not quite, to the midrib. Clasping auricles, from which the name is derived, are usually present in this form.

f. Sessilifolia derivatives, of which the leaf of *macrophylla* may be taken as the type. These derivatives are non-petiolate, as the name indicates.

a. Stenophylla derivatives

Type 1, as may be seen from the drawing of F_2 (cf. pl. 63), seems very close to *angustifolia,* and had light pink flowers. There were 25 plants of $12F_3H_2P_7P_{49}$, the leaves of which were carefully noted; but one passed its flowering stage too early to be judged, so that the colors of the flowers of 24 only are known. Sixteen plants had stenophylla leaves very nearly of type 1, but the extent of the wing structure varied somewhat. The remaining 9 had lanceolata leaves of type 14. In flower color the segregation ratio noted was 5 red : 19 pink. No further generations of this line were grown.

Type 17 was not selected for illustration in F_2, but was a plant very close to *angustifolia.* It had, however, somewhat narrower leaves and deep pink flowers. The 25 plants of $12F_3H_4P_{35}P_{27}$ were uniform and like the F_2 parent except as to flower color; 3 were red and 22 were deeper or lighter pink. This line was not grown through further generations.

Type 21, also, was not selected for illustration of the F_2 plant, but was chosen later for perpetuation because of its extremely close agreement with *angustifolia.* The F_3, $12F_3H_4P_4P_{29}$, consisted of 25 vigorous plants which seemed to be uniform to the finest detail and agreed in every respect with plants of *angustifolia.* There were noticed in the plants of this family peculiar fimbriae attached to the corolla, or split corollas, or, in one instance, a split hose-in-hose flower such as occurs at times also in pure *angustifolia.* This type seemed to be a pure recombination of the characters of *angustifolia.*

Type 21 was continued through to the seventh generation and found to be constant in the uniformity of the individuals in the several families. In 1913, 94 plants of F_4 were grown; in 1914, 85 plants of F_5; in 1915, 10 plants of F_6; and in 1916, 8 plants of F_7. All these populations closely resembled one another as to individuals as well as those of the F_3 and the F_2 parent. They are all so close to *angus-*

tifolia as to be practically indistinguishable from it. This line may be regarded, therefore, as a stable derivative very closely approximating *angustifolia* in all its characters.

b. Latifolia derivatives

Type 2 (cf. pl. 64) approached in F_2 fairly near to some of the variations of F_1 (cf. pl. 62). It might be regarded as a STENOPHYLLA derivative possessing an unusually luxuriant development of the wing of the petiole, but it seems more reasonable to classify it as a LATIFOLIA derivative exhibiting marked narrowing at the base of the lamina, such as is shown in LANCEOLATA derivatives. The flower was designated as light pink in the notes taken at the time of flowering. The leaves of F_3 showed segregation through a considerable range, 6 being close to the LATIFOLIA type of F_1, 6 to the LATIFOLIA type of its F_2 parent (type 2), 8 were SESSILIFOLIA leaves approaching in type those of *macrophylla,* and 4 were AURICULATA leaves of type 10. In 13 the flowers were noted as light pink, in 11 as pink, and in 1 as red. No subsequent generations of this population were grown.

Type 3, in F_2, had (cf. pl. 65) a distinctly ovate lanceolate leaf with a short fairly broadly margined petiole. The flowers were light pink. F_3, $12F_3H_2P_3P_{14}$, amounted to 14 germinations, all of which developed, although slowly, into healthy, normal appearing plants. The flowers were all the light pink color of the F_2 parent, agreeing with those of *angustifolia*, but varying somewhat in shape and size. The leaves were of various shapes, 4 were distinctly petiolate, while 10 were sessile. Of the 4 petiolate plants the petiole of 1 was naked and of 3 more or less winged. Of the 10 SESSILIFOLIA plants, 9 were very similar to type 11, but 1 was rather longer and narrower, although otherwise approaching the same general shape. No further generations from this line were grown.

Type 5 in F_2 (cf. pl. 67) resembled F_1 most nearly, but the winged petiole was short and the corolla tube slightly stouter. The flower color was pink. Of $12F_3H_4P_{41}P_{14}$, 24 plants were grown. Of these 23 had leaves almost exactly like those of the F_2 parent, but 1 had leaves more nearly like AURICULATA of type 8 (cf. pl. 70). Of the 24 plants, 16 had flowers of various shades of pink while 8 had red flowers. This line was not grown in subsequent generations.

Type 6 was represented in F_2 by a plant which resembled F_1 in having a winged petiole to the leaf and a pink flower. It is well repre-

sented on plate 68. In the 25 plants of $12F_3H_4P_2P_{18}$, the height, habit, flower shape, and flower color were close to, if not identical with, those of the F_2 parent. As respects leaf base characters, 14 were LATIFOLIA of type 6, 4 had long, naked petioles, 3 had short, naked petioles, 1 had a long, winged petiole, and 4 were AURICULATA plants nearly of type 10. This seems like a considerable segregation, but the leaves are of only two generic types, viz., petiolate and non-petiolate. In subsequent generations selection was made in one line for LATIFOLIA leaves of type 6, and in the other for AURICULATA leaves of type 10, called type 6a to indicate its derivation.

Of type 6 as thus established 100 plants of F_4 were grown in 1914; 100 plants of F_5 (50 each from 2 different parents) in 1915; 20 plants of F_6 in 1915; and 20 of F_7 in 1916. All the individuals thus grown were constant to LATIFOLIA of type 6 as originally selected.

Of AURICULATA of type 6a, similarly, 100 plants of F_4 were grown in 1913; 94 of F_5 in 1914; 20 of F_6 in 1915; and 20 of F_7 in 1916. All these plants were uniform and true to AURICULATA of type 6a, very close to AURICULATA of type 10. In both these types we have definitely obtained stable recombinations of germinal elements exhibiting characters different from those of the parents.

F_3 of type 7, $12F_3H_2P_{13}P_{48}$ (cf. pl. 69), consisted of a family of 25 plants, all vigorous except one (P_{17}), which was set out in the field later and developed into a "runt," as often happens with such later plantings. All the plants agreed well with one another in height and habit except the "filler," and all agreed in inflorescence, flowers, and leaves. There were some variations in size and lobing of the limb of the corolla, indicating possibly minor segregation, but in all general characters there was uniformity to a large degree. The plants agreed well in all characters with the F_2 parent, and also with the F_1 parent. The color of the flower was light pink, the petioles of the leaves varied somewhat in length, were distinctly and more or less broadly winged, and the blade was heart-shaped, at least at the base in the lower leaves. In all respects these characters were no more variable than they were found to be in F_1.

Type 7 continued to breed true in subsequent generations. It was grown in 1913 (100 plants, F_4), 1914 (2 families of 50 plants each, F_5), 1915 (10 plants, F_6), and 1916 (10 plants, F_7). All were uniform as to leaf and flower color. Type 7 is very close to the type of F_1 and to type 6 described above. It, too, evidently represents a stable recombination of germinal elements derived from both parents.

Type 9 resembles type 5, but had in F_2 a very short winged petiole and elliptical lanceolate blade. It also had pink flowers. F_3, $12F_3H_4P_{41}P_8$, consisted of 25 plants, 18 of which showed LATIFOLIA leaves of type 9, but 7 had SESSILIFOLIA leaves of type 14 (cf. pl. 76). Twenty-one had pink (or light pink) flowers and 4 had red. No further generations of this line were grown.

Type 19 was an F_2 plant of which no drawing was made, but it resembled F_1 (cf. pl. 62), having broadly ovate leaves with a long and broadly winged petiole and pink flowers. F_3, $12F_3H_4P_{35}P_{43}$, consisted of 25 plants, 6 of which had SESSILIFOLIA leaves of type 16 (cf. pl. 78) or nearer, perhaps, to those of *macrophylla,* while 19 had LATIFOLIA leaves of type 19. In 5 plants the flowers were a somewhat darker red than they were in the other 20. This line was not followed further.

c. LANCEOLATA derivatives

Type 13 is similar to type 12 described below, but the leaves of the F_2 plant were more lanceolate and broader and the flowers were lighter pink. The 25 plants of $12F_3H_2P_3P_{44}$ were uniform and like F_2 except in flower color. Four were red, 19 decidedly pink, and 2 inclined to light pink. The line was not grown in subsequent generations.

d. LARIIFOLIA derivatives

Type 12, as shown in plate 74, differed very decidedly in leaf shape from either parent. The long linear-lanceolate leaf had the long tapering curved tip of *angustifolia,* but the blade tapered below, making practically a new type. The flowers were like those of *angustifolia* in shape but were pink. The 25 plants of F_3, $12F_3H_4P_{41}P_{12}$, were exact duplicates of F_2 as to habit, leaf, and flower shape, but 10 had red and 15 had pink flowers of various shades, mostly dark. None seemed as light pink as *angustifolia.*

This is the most interesting of the types carried through subsequent generations, representing, apparently, a new combination of leaf characters. One of the pink flowering F_3 plants was chosen for seed and the designation, type 12, retained for this and its progeny, while the designation, 12a, was given to one of the red flowering F_3 plants also chosen for seed.

Type 12, as thus limited to the pink flowered plant, gave scanty germination and few plants for F_4 in 1913. Apparently it was still varying slightly in color within the pink shades, although fairly uni-

form except for one aberrant (?) plant of a decidedly lighter shade. Two "normal" parents of F_4 gave 88 and 100 plants of F_5 in 1914, which were uniform and of a bright pink color. In 1915 F_6 showed 10 plants, and in 1916 F_7 also showed 10 plants, still uniform and pink.

Type 12a on being segregated in the second growing of F_3 in the season of 1913 yielded 100 plants of F_4 in 1914, uniform and of deep red flower color. F_5, of 10 plants in 1915, and also F_6, of 10 plants in 1916, produced uniform individuals of deep red flower color.

We find, then, in types 12 and 12a definite fixations of the LORII-FOLIA type, one with uniformly pink flowers and one with uniformly deep red flowers.

e. AURICULATA derivatives

Type 8 is represented in F_2 by a plant which had a leaf with an extremely constricted base (cf. pl. 70) and deep red flowers. It is not a typical AURICULATA derivative, but is included under this heading because it resembles the members of this class more closely than those of any other. F_3, $12F_3H_2P_3P_{41}$, consisted of 25 plants which were uniform in height, habit, and flower color, and in agreement with F_2 in these respects. The leaves, however, were of two distinct types, 16 AURICULATA of type 8 and 7 SESSILIFOLIA of type 16 (cf. pl. 78), the latter being near to the type of *macrophylla*. No further generations of this line were grown.

Type 10, as shown by the drawing (pl. 72), had a peculiar leaf, near to the *macrophylla* type, yet deeply constricted at the base into a narrow and extremely abbreviated structure which may resemble a petiole or only a deeply constricted blade. There were, however, auricles partially clasping the stem and slightly decurrent. The leaf form was that characteristic of *N. Tabacum* var. *macrophylla purpurea* (cf. Setchell, *loc. cit.*). All 24 plants of F_3 had the same type of leaf as F_2, but the flowers were of three fairly readily distinguishable shades; 3 were red, 16 pink, and 5 light pink. F_2 had very dark pink flowers. The line was not grown in further generations.

Type 6a is a true AURICULATA derivative which segregated in F_3 from an F_2 LATIFOLIA selection. Its occurrence and behavior are described in connection with the account of type 6, the LATIFOLIA type from which it segregated. Grown in the pure line for five generations it has remained constant for the AURICULATA type of leaf.

f. Sessilifolia derivatives

Type 11 (cf. pl. 73) in F_2 gave 25 plants in $12F_3H_4P_{41}P_9$, all vigorous except one, but that one showed the same characters of leaf and flower as the others. All 25 plants possessed a sessilifolia type of leaf very close to the F_2 parent and uniform among themselves. There were two distinct shades of color of the flowers, 9 red and 16 pink. No further generations of this line were grown.

Type 14 (cf. pl. 76), so far as F_2 is concerned, was one of those having sessile leaves of a broadly lanceolate type and pink flowers. There were 25 plants in $12F_3H_2P_3P_{38}$, 24 had sessilifolia leaves of type 14, while one (a "filler") had auriculata leaves like type 8; 19 had pink (or light pink) flowers, while 6 had red flowers. This line was not followed through subsequent generations.

Type 15 (cf. pl. 77) was represented in F_3, $12F_3H_2P_3P_{10}$, by 25 vigorous plants which seemed surprisingly uniform and approached *macrophylla* very closely as to leaf and color of the flower. In the flower, however, the color seemed even darker than that of *macrophylla*, there were only slight traces of the white triangular markings on the limb, the limb was much more deeply lobed, and the tube less stout and with the infundibulum much less abruptly swollen. These differences seem to indicate that type 15, which all the F_3 plants closely resemble, is not an exact recombination representing *macrophylla*.

Type 15 was represented in 1913 by two families, F_3 of 10 plants and F_4 of 100 plants. Both families were uniform as to individuals, and agreed with the F_3 population grown in 1912 as well as with the F_2 ancestor of the season of 1911. As this line seemed to be constant and very close to, although not absolutely identical with, *macrophylla*, differing in flower shape and leaf shape to some extent, type 15 was considered to be a fixation and no further cultivation of it was made.

Type 16 (cf. pl. 78), which in F_2 approached *macrophylla* very closely in leaf, flower shape, and flower color, was represented in F_3, $12F_3H_2P_3P_8$, by 25 plants. These were all alike and closely resembled the F_2 parent in all respects except in leaf shape. Fifteen had sessilifolia leaves of type 16 while 8 had auriculata leaves approaching those of type 10 (cf. pl. 72). This line was not grown in further generations.

Type 18 is the designation given to an F_2 plant, of which no drawing was made. It seemed close to *macrophylla*, but the flower color

was pink and the leaves were more slightly attenuate at the base. F_3, $12F_3H_4P_{35}P_{38}$, gave 25 plants, 13 of which had the SESSILIFOLIA leaf of type 18; 7, AURICULATA of type 10; and 5, AURICULATA of type 8. In flower color, 17 were some shade of pink and 8 red. The line was not grown in further generations.

Type 20 was not selected for illustration in F_2, but was a plant chosen because of its very close resemblance to *macrophylla*, coming even closer than type 16. The F_3, $12F_3H_4P_{40}P_{44}$, consisted of 25 vigorous plants of remarkable uniformity. In height, habit, inflorescence, flower, color, shape, fruit, etc., the details follow those of *macrophylla* so closely as to be indistinguishable unless possibly by careful and laborious biometric study. This type may represent a practically pure recombination equivalent to *macrophylla*, and is to be compared and contrasted with type 15.

In 1913 two families of F_4, one of 21 plants and the other of 100 plants, were uniform, as were 3 families of 50 plants each of F_5 in 1914. In 1914, however, a surprising thing happened. A fourth family of F_5, consisting of 50 plants, was uniform except one plant which had pink (instead of red) flowers and an AURICULATA leaf approximating type 8 or 10. It seems certain that this plant must have been an intruder, but its seed was saved under bag and grown and is noted below and on the pedigree chart as type 20a. The other 3 plants of F_5 whose seed was sown in 1915 gave type 20 in F_6 in families of 10, 9, and 8 respectively, and in turn the seed of 4 individuals of ''pure'' type 20 gave, in 1916, uniformity in families of 10 each.

Type 20a, which originated or intruded in 1914, in one plant of F_5 of type 20 gave in F_6, in 1915, 10 plants segregating for flower color and probably also for leaf characters, although the notes taken are inconclusive on the latter point. In 1916 F_7 of 10 good plants showed uniformly red flowers, but 7 had SESSILIFOLIA leaves, 4 of which were decidedly contracted at the base and 3 had very short winged petioles (AURICULATA of type 8 or type 10). On the whole it seems most likely that the single plant in the F_5 family was an intruder, since all other families of the line have been constant since F_2. A stray seed somewhere along the processes of culture would explain it and its appearance is all the more incomprehensible as a matter of inclusion in the pedigree of type 20, as it is so close to *macrophylla* as to seem practically identical with it.

5. SUMMARY OF FLOWER COLOR OBSERVATIONS IN F_2 AND SUBSEQUENT GENERATIONS

In tables 3 and 4 we have summarized the numerical data with respect to flower color inheritance in F_3 and in the subsequent populations. In table 3 are assembled data with respect to the behavior of red flowering selections from populations segregating for red and pink. It will be noted that all the five selections which were made bred true for red flower color in the succeeding generations. In table

TABLE 3

INHERITANCE OF RED FLOWER COLOR IN F_3, *et seq.*

Type Numbers	Garden Numbers	Flower color of population
8	$12F_3H_2P_3P_{41}$	25 red
12a	$14F_4H_4P_{41}P_{12}P_1$	100 red
15	$12F_3H_2P_3P_{10}$	25 red
16	$12F_3H_2P_3P_8$	24 red
20	$12F_3H_4P_{40}P_{44}$	25 red

TABLE 4

POPULATIONS FROM PINK FLOWERING SELECTIONS OF ALL SHADES IN F_3 *et seq.*

Type Num- bers	Garden Numbers	Parent Color	Flower color classification		
			red	pink	light pink
3	$12F_3H_2P_3P_{14}$	light pink	....	....	14
6	$12F_3H_4P_2P_{18}$	pink	....	25	... .
7	$12F_3H_2P_{13}P_{48}$	light pink	....	....	25
12	$13F_4H_4P_{41}P_{12}P_8$	pink	....	88	....
12	$13F_4H_4P_{41}P_{12}P_9$	pink	....	100	....
21	$12F_3H_4P_4P_{29}$	light pink	....	... .	25
1	$12F_3H_2P_7P_{49}$	light pink	5	19	(or light pink)
2	$12F_3H_2P_3P_{30}$	light pink	1	24	(13 light pink)
5	$12F_3H_4P_{41}P_{14}$	pink	8	16	(or light pink)
9	$12F_2H_4P_{41}P_8$	pink	4	21	(or light pink)
10	$12F_3H_4P_{41}P_{17}$	pink	3	21	(5 light pink)
11	$12F_3H_4P_{41}P_9$	pink	9	16	(0 light pink)
12	$12F_3H_4P_{41}P_{12}$	pink	10	15	(0 light pink)
13	$12F_3H_2P_3P_{44}$	pink	4	21	(2 light pink)
14	$12F_3H_2P_3P_{38}$	pink	6	19	....
17	$12F_3H_2P_4P_{35}P_{27}$	pink	3	22	....
18	$12F_3H_4P_{35}P_{38}$	pink	8	17	....
19	$12F_3H_4P_{35}P_{43}$	pink	25	....	(5 darker red)
Totals of segregating populations			60	187	(Types 2 and 19 excluded)

4 are assembled the data from pink flowering selections from populations which showed segregation into red and pink. In this table the populations which bred true for pink are assembled in the upper portion of the table, and those which showed further segregation into red and pink are assembled in the lower portion. Of the 18 selections made, 7 bred true for pink (or light pink), and 10 gave segregation in the succeeding generation in about the ratio of 3 pink : 1 red. The total figures for the 10 populations—187 pink : 60 red—are in very satisfactory agreement with the simple Mendelian ratio. The family of type 19 behaved in an anomalous fashion, which may indicate misclassification of the F_2 parent; and the family of type 2, which showed only one red plant has been included among those which bred true for pink. Strictly light pink selections should have given only light pink flowers in subsequent generations; the pink ones should all have given segregating populations. The evidence indicates that this result would be obtained if segregation occurred for *only one pair of allelomorphs*. The difficulty, in part at least, appears to be the result of segregation of modifying factors in the populations. These factors apparently have an effect on flower color sufficient to obscure segregation into pink and light pink, but not enough to obscure the segregation into red and pink. The actual results indicate an approximate agreement with expectation, but the breeding test clearly is necessary in order to determine the actual distribution of the pink individuals into their genetic classes.

6. LATER SOWINGS OF F_2 AND F_3 OF THE ANGUSTIFOLIA– MACROPHYLLA SERIES

In 1916 and 1917 certain families of F_2 and F_3 of H_2 were grown in order to reëxamine them in the light of data previously collected and to determine whether or not any more definite classifications could be made than those stated in the preceding pages. The populations grown are described briefly below.

$16F_2H_2P_6$, as the population number would indicate, was a sowing of seed of $10F_1H_2P_6$ from the original F_1 population of H_2. As in previous cases, the segregation as regards leaf shape was so complex as to preclude definite classification. The types previously noted for second generation populations were all in evidence and along with them practically every sort of intermediate. The height of plants and general habit likewise agreed with the description previously given.

It was possible as in previous instances to segregate the plants into definite flower color classes. In order to make this segregation as accurate and free from bias as possible a special method of classification was adopted. At the height of the blooming season, single typical flowers were collected from each plant of a population and placed in vials correspondingly numbered. These specimens were then taken into the laboratory, where they could be classified under optimum light conditions. The specimens so collected could then be shifted around into their phenotypic classes and properly compared with each other and with the parent colors. The color classification thus obtained was individually recorded, and later the population was checked over in the field to insure correction of any errors of classification. The surprising feature of this population was a sharp, three-class segregation into red, pink, and light pink; the reds the shade of *macrophylla,* the light pinks almost exactly that of *angustifolia,* and the pinks intermediate between the two. Within the classes there appeared to be no significant differences in depth of shade. Two plants bore no flowers. The ratio obtained was 15 red : 23 pink : 10 light pink.

$16F_2H_2P_{17}$ was likewise a sowing of the seed of one of the original F_1 plants, in this instance of $10F_1H_2P_{17}$. As respects habit, height, and leaf shape, there was a strict resemblance throughout of this population to the one described above. Flower color was studied in the same manner and with substantially the same results. However, in this population there was a shading off from pink to light pink, such that it was impossible to draw a sharp line between these two classes as was done in the previous population. The shading off was abrupt, but there were, nevertheless, a few plants on the border line. The observed ratio was 16 red : 34 pink and light pink.

In 1917 six F_3 populations, each containing approximately 100 plants, were grown in order to make further studies of the inheritance of leaf shape. It was impossible, however, to study these plants as thoroughly as might have been desired on account of conditons obtaining during 1917. However, specimens of leaves from each plant were pressed and preserved and these were studied and classified in the summer of 1919. A brief account of each population follows:

$17F_3H_2P_{17}P_6$ was a sowing from $16F_2H_2P_{17}P_6$, a STENOPHYLLA selection. With respect to leaf base characters the segregation was roughly but rather obviously into two types, a long petioled STENO-PHYLLA class approximating type 1 in appearance, and an AURICULATA

class approximating type 10. Within the STENOPHYLLA class there was a variation in the amount of ''wing'' on the petiole and in the type of blade base, some having the abrupt base of type 1, whereas others had an attenuated type of blade which gradually drew in to the petiole. In the AURICULATA class there was also a variation from the strict form of type 10 to a type which lacked the flaring auricle typical for that form, and had a very short naked petiole. In addition to this variation in the amount of ''wing'' of the constricted class there was also a difference in the presence or absence of attenuation noted for the STENOPHYLLA class, some plants having leaves abruptly drawn in to the midrib, whereas others were very markedly attenuated. The difference in this respect appeared to be equivalent in the two distinct classes, i.e., it was independent of any difference in the ''petioled'' or ''constricted'' condition. With respect to STENOPHYLLA vs. AURICULATA the segregation was 66 STENOPHYLLA : 32 AURICULATA.

$17F_3H_2P_{17}P_8$ was a sowing from $16F_2H_2P_{17}P_8$, another STENO-PHYLLA selection. The leaf classes obtained here were two, STENO-PHYLLA (type 1) and SESSILIFOLIA (type 15). The segregation into the two classes was distinct, but, as in other populations, there was a great deal of variability in each class. There was attenuation of the kind previously noted in both classes. Some of the petioled individuals had distinct wings, but the larger number were naked. Some few individuals had very short petioles. The segregation ratio was 76 STENOPHYLLA : 24 SESSILIFOLIA.

$17F_3H_2P_{17}P_{19}$ was a sowing from $16F_2H_2P_{17}P_{19}$, a STENOPHYLLA selection. The population was remarkably uniform in leaf shape, which closely approximated *angustifolia* with minor differences. The straplike leaves which are a characteristic feature of the upper portions of plants of *angustifolia* were lacking in this population, and the leaf tip and distal portion of the leaf blade did not narrow so gradually in this population as in *angustifolia*. Otherwise, the characters of the plants throughout were closely similar to *angustifolia*.

$17F_3H_2P_{17}P_{22}$ was a sowing of seed of $16F_2H_2P_{17}P_{22}$, a STENOPHYLLA selection. Of the six F_3 populations studied, this one exhibited the greatest diversity in segregation. With respect to leaf base characters, there were two outstanding classes, STENOPHYLLA and SESSILIFOLIA (type 15), which could be separated readily. Within the STENOPHYLLA class, however, most of the individuals exhibited a more or less winged condition. Within the SESSILIFOLIA class, on the other hand, most of the individuals exhibited more or less narrowing of the leaf base, like

type 14. A few of the sessile individuals, instead of exhibiting gradual and uniform narrowing toward the base of the leaf, were constricted to a degree intermediate between AURICULATA of type 10 and SESSILI-FOLIA of type 15. With respect to STENOPHYLLA versus SESSILIFOLIA the observed segregation was 67 STENOPHYLLA : 32 SESSILIFOLIA.

$17F_3H_2P_{17}P_3$ was a sowing of seed of $16F_2H_2P_{17}P_3$, an F_2 SESSILI-FOLIA selection. The leaves throughout had the sessile type of leaf base characteristic of *macrophylla,* but there were many modifications of it in the population. A rough classification with respect to these modifications of the *macrophylla* type of leaf base gave the following results:

On 59 plants, the leaf bases were very nearly the form typical for *macrophylla.*

On 22 plants, the leaf bases were gradually attenuated toward the base, resembling LANCEOLATA of type 13 as a mean. This attenuated form of the sessile leaf was a very striking feature of this population.

On 10 plants, the leaf bases were intermediate in type between LANCEOLATA of type 13 and the typical *macrophylla* form.

On 2 plants, the base of the leaf immediately above the point of attachment was noticeably constricted, the leaf base thus formed being intermediate between the *macrophylla* type and AURICU-LATA of type 10.

On 2 plants, the leaves were intermediate in constriction of the leaf base between the strict *macrophylla* type and that of the two plants described immediately above.

The classification here given is presented only to show that the sessile type of leaf base characteristic of *macrophylla* is subject to a number of very definite modifications which probably account for some of the complex types of segregation observed in other populations.

$17F_3H_2P_{17}P_{12}$ was a sowing from $16F_2H_2P_{17}P_{12}$, a SESSILIFOLIA selection. With respect to leaf base segregation there were two distinct classes, SESSILIFOLIA (type 15) and AURICULATA (type 10). There was here also a marked degree of variation within the classes. Within the sessile class the variation was in amount and kind of narrowing of the leaf blade toward the base. A few plants showed a condition approaching the AURICULATA type in this respect, whereas others showed a gradual attenuated form of narrowing such as has been noted before in other populations. Within the AURICULATA class most of the individuals instead of possessing the slight wing and flaring

auricles of type 10 had short naked petioles. A few were strictly of type 10. The following segregation ratio was noted: 61 SESSILIFOLIA : 27 AURICULATA.

7. CROSSES OF DERIVATIVES WITH THE PARENTS

In the preceding account we have pointed out that by growing definite hybrid selections in the pure line through a number of generations it has been possible to establish a certain number of stable derivatives which represent more or less obvious recombinations of characters of the original parents. In a Mendelian sense, they represent stable reorganized germinal complexes containing hereditary elements that have been derived from both parents. Obviously such recombinations of Mendelian units must differ in fewer units from either parental type than did the parental types from each other. To test some of these derivatives we have crossed them with the original parents, usually with the one to which they bore the closest resemblance, in order to observe how complex a type of segregation the hybrids thus obtained would exhibit as compared with that of the original *angustifolia-macrophylla* hybrids. In so far as they have been studied to date, a description of these hybrids and their progenies follows:

SESSILIFOLIA×*macrophylla.* F_5 SESSILIFOLIA of type 20 was crossed with *macrophylla* giving $H_{50} =$ type 20♀ × *macrophylla*♂ and $H_{51} =$ reciprocal thereof. The derivative parent here very closely resembles *macrophylla* throughout in flower color and shape, habit, leaf shape, etc. $15F_1H_{50}$ and $15F_1H_{51}$, two families of 50 plants each, were equivalent in every respect. The plants were very close indeed to *macrophylla,* as is also the SESSILIFOLIA parent. The only difference readily observed was some variation in the amplitude of the corolla. In H_{51}, a plant with a larger and one with a smaller corolla were selected for pure seed. In F_2, grown in 1916, one family of H_{50} and two families of H_{51}, of 50 plants each were grown. The flower color in the F_2 populations was throughout that of *macrophylla* and the leaf characters also were those of *macrophylla.* All three families were remarkably uniform, not only agreeing with one another but uniform as to indivduals. They all resembled closely the *macrophylla* type and there was no definite segregation of any kind in them. The three populations appeared to be replicas of *macrophylla* throughout except that they were slightly more robust.

Latifolia × *angustifolia*. F_5 Latifolia of type 6 was crossed with *angustifolia* giving H_{52} = type 6♀ × *angustifolia*♂ and H_{53}, its reciprocal. The derivative parent possessed the short winged petiole characteristic of Latifolia of type 6. In F_1 50 plants of each cross were grown. They exhibited the long naked petiole characteristic of *angustifolia*.

In F_2 two populations of 50 plants each were grown. In color of flowers the two populations were light pink throughout, closely corresponding in this respect to *angustifolia*. In leaf shape the segregation was sharply into two classes: the Stenophylla type of leaf base (long, naked petiole) and the Latifolia type (shorter, winged petiole). There was some variation in the Stenophylla class suggesting intermediacy between *angustifolia* and Latifolia, but the forms exhibiting it showed a graded series from strict Stenophylla to intermediate. The Latifolia class was very uniform and sharply set off from the other class. The segregation ratios observed were as follows:

	Stenophylla	Latifolia
$16F_2H_{52}P_{16}$	42	8
$16F_2H_{53}P_{35}$	36	14
	—	—
Totals	78	22

Auriculata × *macrophylla*. F_5 Auriculata of type 6a was crossed with *macrophylla* giving H_{54} and H_{58} = F_5 type 6a♀ × *macrophylla*♂ and H_{55} and H_{59}, their reciprocals. It should be observed that type 6a is an early segregant from the Latifolia of type 6 of H_{52} and H_{53}. In F_1 50 plants were grown of each of the four parents. All four populations were equivalent in every respect. All the plants had pink flowers, although one plant had flowers of a lighter shade than the others, and leaves of a shape somewhat intermediate between the two parents, i.e., they were more contracted at the base than *macrophylla*, but much less so than those of type 6a. One plant of H_{55}, namely $15F_1H_{55}P_{16}$, showed larger corollas than any of the other F_1 plants of any family, and was selected for further breeding.

In F_2 four families were raised and they proved to be equivalent in all respects, except as noted. There was sharp segregation for leaf shape into the Sessilifolia and the sharply constricted Auriculata type. In the Sessilifolia class there were a number of obvious intermediates, as might be expected from the characters exhibited by F_1, but they formed a continuous series with the strict Sessilifolia forms. The Auriculata class did not intergrade with the dominant class.

Segregation for flower color was studied by the method described above. The color distinction between red and pink was sharp and easily drawn. In the pinks, however, there was a continuous series of shades from the deep rose pink characteristic of F_1 to the light pink typical for *angustifolia*. Numerical data are given in table 5.

TABLE 5

F_2 SEGREGATION OF PINK AURICULATA $\times$ RED SESSILIFOLIA.

Garden Numbers	Pink sessilifolia	Red sessilifolia	Pink auriculata	Red auriculata
$16F_2H_{54}P_7$	32	6	9	3
$16F_2H_{55}P_{16}$	23	11	11	5
$16F_2H_{55}P_{29}$	26	13	7	4
$16F_2H_{58}P_{26}$	26	11	7	2
$16F_2H_{59a}P_{35}$	26	12	6	2
Totals	133	53	40	16
Expected	136	45	45	15

In 1918 in connection with flower size studies three more populations of F_2H_{55} were grown. Leaves were collected from each plant and pressed, and leaf shape studies were made on these preserved specimens. The studies were not so satisfactory as those made in the field, where it is possible to examine all the leaves on a given plant; nevertheless, the data derived from the studies agreed substantially with those obtained in 1916 from field studies. It was noted in these studies that there was a distinct class of "attenuated" leaves similar to those which have been described in previous populations. Both attenuation and constriction were observed to occur in the leaves of some individuals, and this gave rise to some difficulty in classification. Numerical data are given in table 6.

TABLE 6

F_2 SEGREGATION OF SESSILIFOLIA $\times$ AURICULATA.

Garden Numbers	SESSILIFOLIA	AURICULATA
$18F_2H_{55}P_{40}$	67	28
$18F_2H_{55}P_{41}$	79	18
$18F_2H_{55}P_{46}$	79	17
Totals	225	63

Stenophylla × *angustifolia*. Reciprocal crosses were made between F_5, stenophylla of type 21 and *angustifolia;* $H_{56} = F_5$ type 21♀ × *angustifolia*♂ and H_{57}, its reciprocal. Stenophylla of type 21 has been described previously as a stable derivative closely approximating *angustifolia* in all its characters. F_1 families of 50 plants of each hybrid were raised in 1915. They were uniform throughout and so close to *angustifolia* in all characters as to be indistinguishable from it. One plant seemed to be of a slightly darker pink corolla color. $15F_2H_{56a}P_{11}$ was the only F_2 family raised. The flower color of this population was about the shade of *angustifolia* and uniform throughout the population. The family showed only a slight variation in the base of the blade such as is also seen in populations of *angustifolia*.

8. DISCUSSION OF RESULTS OF THE ANGUSTIFOLIA–MACROPHYLLA SERIES OF INVESTIGATIONS

Obviously the outstanding result of this series of investigations of hybrids between *angustifolia* and *macrophylla* is a demonstration of the complexity of the germinal differences which exist between the two varieties with respect to practically every character contrast which may be made between them. Only in one instance, the contrast between the light pink flower color of *angustifolia* and the red of *macrophylla,* is a simple Mendelian formulation possible. Here evidently the main flower color difference is dependent upon a simple allelomorphic contrast. Red × light pink gives F_1 intermediate pink, and F_2 1 red : 2 intermediate pink : 1 light pink. The red segregants breed true for red, the light pinks for light pink, and pink continues to segregate in the typical mono-hybrid fashion. Inasmuch as the intermediate pinks and light pinks form an intergrading series, it is convenient to look upon red as the recessive color. Accordingly we give this pair of factors the designation, **Rr,** following the mnemonic system advocated by Morgan, and shall so refer to it in what follows. The difficulty among the pinks appears to be due not only to phenotypic variation but also to the existence of modifying factors which have a relatively slight effect upon flower color expression. These less striking modifications of flower color we are seeking to analyze further.

In the leaf shape investigations, the complexity of the results is plainly evident from an examination of the data presented in the foregoing pages. Although the behavior here is complex, in every feature it parallels the Mendelian expectation for complex factor relations.

In F_2 the variety of leaf shapes encountered was nothing short of bewildering and series could be built up from them showing complete intergradation from one type to another. Selection of phenotypes from F_2, however, gave F_3 populations in which the complexity of segregation was usually reduced in a very definite fashion. Most of the populations exhibited fewer classes than F_2, and the selection of F_2 phenotypes held the expression of F_3 within very definite limits. Thus selection of SESSILIFOLIA forms gave in F_3 either all SESSILIFOLIA or approximately 3 SESSILIFOLIA : 1 AURICULATA. In no case did such selections give F_3 populations with STENOPHYLLA or LATIFOLIA leaf types. A summary in detail of the type of populations produced is as follows.

STENOPHYLLA selections may segregate in a variety of ways. Thus, type 1 showed approximate segregation into 3 STENOPHYLLA : 1 SESSI-LIFOLIA. Type 21 bred true to the STENOPHYLLA characters. Among STENOPHYLLA selections grown in 1917, population $17F_3H_2P_{17}P_6$, showed approximate segregation into 3 STENOPHYLLA : 1 AURICULATA; $17F_3H_2P_{17}P_8$, 3 STENOPHYLLA :1 SESSILIFOLIA; $17F_3H_2P_{17}P_9$ bred true for STENOPHYLLA, and $17F_3H_2P_{17}P_{22}$ gave a rather indefinite segregation of approximately 3 STENOPHYLLA : 1 SESSILIFOLIA. LATIFOLIA derivatives crossed with *angustifolia* gave F_1 STENOPHYLLA and F_2 approximately 3 STENOPHYLLA : 1 LATIFOLIA.

LATIFOLIA selections also segregate in perplexing fashion. The F_1 population of *angustifolia* × *macrophylla* is typically LATIFOLIA in its characters. LATIFOLIA under certain conditions therefore is a very complex hybrid expression. Recurrence of complex segregation of a LATIFOLIA selection is shown in F_3 of type 2. F_3 of type 3 exhibited a rather anomalous segregation ratio of petioled and sessile forms. Type 5 apparently bred true, although there was one anomalous plant in the population. Type 6 exhibited complex segregation, with an indication of a ratio of 3 LATIFOLIA : 1 AURICULATA; with subsequent establishment of both LATIFOLIA and AURICULATA in constant races. Type 7 bred true for a type of leaf like F_1; and type 9 gave approximate segregation of 3 LATIFOLIA : 1 SESSILIFOLIA.

LORIIFOLIA and LANCEOLATA derivatives are really variations of the SESSILIFOLIA type. They were both produced in constant races. Their genetic relation to the other forms is, however, not well established by this series of investigations. Although these two are really quantitative variations from the strict SESSILIFOLIA type, nevertheless, certain of our data indicate discontinuous inheritance of these contrasts.

The same quantitative factors that differentiate the narrow-leaved forms of SESSILIFOLIA from the typical broad-leaved forms may apparently differentiate narrow-leaved STENOPHYLLA, LATIFOLIA, and AURICULATA forms from the more typical broad-leaved ones. It is of interest in this connection to note that LORIIFOLIA derivatives have much narrower leaves than either of the original parents.

We have been especially interested in these LORIIFOLIA derivatives because they are somewhat like the narrow-leaved forms that Hassel-bring (1912) found among Cuban tobaccos, and which are so well recognized among Cuban growers as to have received the specific designation of *lengua de vaca* or "cow's tongue." Our results indicate that it is possible for such forms to arise by segregation from crosses between broader leaved forms. The *lengua de vaca* of the Cuban growers is, therefore, probably a segregation product which could easily be eliminated by the adoption of proper pure line methods of breeding.

AURICULATA forms appear to breed true whenever segregated. The exception is type 8, which requires further investigation. It may be a leaf type similar to AURICULATA but of different genetic constitution. AURICULATA of type 10 bred true in F_3. The AURICULATA form 6a, which segregated from type 6 bred true thereafter. AURICULATA crossed with *macrophylla*, H_{54}, H_{55}, H_{58}, and H_{59}, gave SESSILIFOLIA in F_1 and in F_2 3 SESSILIFOLIA : 1 AURICULATA.

SESSILIFOLIA forms have broad sessile leaves, the distinguishing feature being merely their sessile mode of attachment. Of such selections from the original F_2 populations, four, with the exception of one anomalous plant, bred true for SESSILIFOLIA. Each of the other three populations segregated into SESSILIFOLIA and AURICULATA in about the ratio of 3 SESSILIFOLIA : 1 AURICULATA. Two SESSILIFOLIA selections were grown in 1917. One of these bred true to SESSILIFOLIA; the other gave 3 SESSILIFOLIA : 1 AURICULATA. The behavior of SESSILIFOLIA in relation to STENOPHYLLA and LATIFOLIA is explained above.

On the basis of these results we may distinguish certain definite allelomorphic pairs of factors as follows

Ss, STENOPHYLLA versus SESSILIFOLIA : **SS** being long petioled like *angustifolia*, and ss broadly sessile like *macrophylla*. The heterozygote may possibly approach an intermediate condition similar to LATIFOLIA.

Ll, STENOPHYLLA versus LATIFOLIA : **LL** being long petioled like *angustifolia*, and ll short petioled like LATIFOLIA and with a distinct but not broad wing. The contrast is really one of **SSLL**, STENOPHYLLA

versus **SSll**, LATIFOLIA. Both **ssLL** and **ssll** are probably typical SESSI-
LIFOLIA forms. Here again the heterozygote probably shows an indis-
tinct type of intermediacy.

   Aa, SESSILIFOLIA versus AURICULATA: **AA** having the broad clasp-
ing leaf base characteristic of *macrophylla,* and **aa** the deeply con-
stricted leaf bases with flaring auricles characteristic of AURICULATA.
The contrast here is really one of **ssAA**, SESSILIFOLIA versus **ssaa**, AURI-
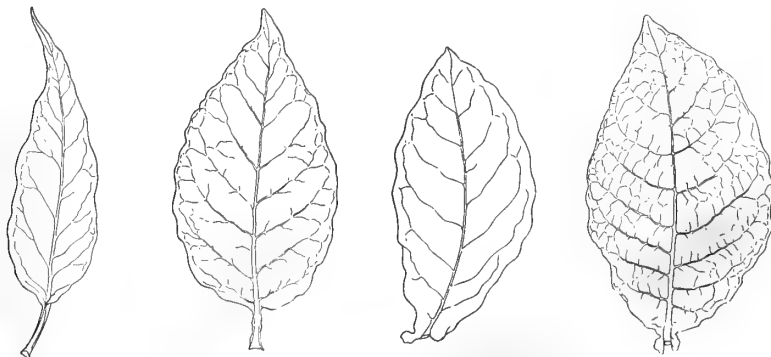CULATA, for these factors are evidently latent when in combination with
SS or **Ss**.

Fig. 2.   Leaf base types of the *angustifolia-macrophylla* series. Left to right:
STENOPHYLLA, LATIFOLIA, SESSILIFOLIA, and AURICULATA.

   Some of the possible genotypes, their phenotypic expression, and
genetic behavior are included in table 7. Here only monohybrid segre-
gation is considered because it is doubtful, on account of the various
types of intermediacy shown by heterozygotes, whether it would be
possible to classify dihybrid and trihybrid populations satisfactorily.

TABLE 7
GENETIC BEHAVIOR OF VARIOUS LEAF TYPE GENOTYPES.

Genotype	Phenotype	Genetic behavior
SSLLAA	STENOPHYLLA	Breeds true
SsLLAA	STENOPHYLLA	3 STENOPHYLLA : 1 SESSILIFOLIA
SSLlAA	STENOPHYLLA	3 STENOPHYLLA : 1 LATIFOLIA
SSLLAa	STENOPHYLLA	Breeds true
SsLLaa	STENOPHYLLA	3 STENOPHYLLA : 1 AURICULATA
SSllAA	LATIFOLIA	Breeds true
SsllAA	LATIFOLIA	3 LATIFOLIA : 1 SESSILIFOLIA
Ssllaa	LATIFOLIA	3 LATIFOLIA : 1 AURICULATA
ssLLAA	SESSILIFOLIA	Breeds true
ssllAA	SESSILIFOLIA	Breeds true
ssllAa	SESSILIFOLIA	3 SESSILIFOLIA : 1 AURICULATA
ssllaa	AURICULATA	Breeds true

Although intermediacy of the heterozygote appears to be the normal thing in these leaf shape contrasts, it is proper to state that this intermediacy may depend to some extent upon the effect of modifying factors rather than upon the heterozygous conditon of a pair of allelomorphs. Thus the intermediate conditon between STENOPHYLLA and AURICULATA is met with in populations which do not contain AURICULATA segregation products. There are so many modifying factors in this series of investigations that it is probably impossible for us to declare definitely that in any one instance our observed segregation was wholly the result of segregation of one pair of allelomorphs. Further investigations are in progress, the purpose of which is to isolate and evaluate, if possible, certain of these subsidiary factors. For the present we can only state our certain knowledge of their existence, and our belief as to their various effects.

IV. CALYCINA–VIRGINICA SERIES

The *calycina-virginica* series of hybrids and derivatives has received much less attention than has been given to the previous series; partly because the differences between the parents are less striking and the diversity of segregation products was not so great. Two hybridizations were made: H_{18} which had *calycina* for the female and *virginica* for the male parent and H_{20} which was the reciprocal cross.

1. PARENTS OF THE CALYCINA–VIRGINICA SERIES

Elsewhere Setchell has given descriptions of *calycina* and *virginica* (''Maryland''). Like *angustifolia* and *macrophylla,* these two varieties possess distinct sets of characters which set them apart from the other *Tabacum* varieties that have been grown in the University of California Botanical Garden.

Calycina is represented in our cultures by a variety, U. C. B. G. 110/05, which was originally received from the Botanical Gardens of Cambridge University. The figure previously published (cf. Setchell, *loc. cit.,* pl. 4) well represents the general habit and type of the plant. The particular features of the characteristic teratological flower of *calycina* are better shown in plate 79, in which the leaf shape is also illustrated in more characteristic fashion. For illustrations of some of the variations which occur in the expression of the split hose-in-hose flowers the reader is referred to Goodspeed and Clausen, 1917, plate 45. The legends to the two figures of this plate should be reversed as indicated in the references to the plate in the text of this earlier article.

In stature, as previously mentioned, *calycina* belongs to the low corymbose group of *Tabacum* forms. In height the central axis usually varies between 60 and 75 cm. The laterals, however, which develop later from the base, overtop the central axis and reach a height of 120 to 135 cm. Like *macrophylla,* central axis and laterals bear close panicles of corymbose racemes, the laterals developing successively from the base. The stems and branches are stouter than those of *angustifolia* and the leaves do not droop so considerably. In these respects *calycina* occupies an intermediate position between *angustifolia* and *macrophylla.*

The leaves of *calycina,* as plate 79 will show, are sessile, but they are distinctly different from those of either *angustifolia* or *macrophylla.* Curiously enough, however, they do rather closely approximate certain of the derivatives of the *angustifolia-macrophylla* series, as, for example, the LANCEOLATA and LORIIFOLIA leaves of types 13 and 14 respectively, illustrated in plates 75 and 76. The leaves vary from broadly to narrowly lanceolate, tapering toward both base and apex, and usually with a long curved tip. The broader leaves are borne at the base of the plant, those above them becoming successively narrower in a continuous series until the linear leaves or straplike bracts of the inflorescence are reached. There are no auricles at the base of the leaf.

The inflorescence is in the form of a very close panicle of racemes, the secondary axes of which are mostly patent, and more or less recurved or bent back. The flower as a whole is of a very characteristic split hose-in-hose type. The corolla is usually split on one side, sometimes twice split, and more or less curved. The characteristic splitting of the corolla is seen even in very young buds and often the pistil protrudes from them. Typically the calyx has an elongated whitish green tube, with 3 to 5 of its tips more or less petaloid. Sometimes strips of petaloid tissue extend down the entire length of the calyx. The pod is ovoid oblong in shape. As it enlarges it splits the calyx, which then withers and drops off like the corolla, leaving a naked, whitish green capsule. The flower color is red fading to bluish purple, apparently the same as that of *macrophylla.*

Virginica is represented by U. C. B. G. 78/05, a strain received from the United States Department of Agriculture under the identification number "205–20–7." It is figured by Setchell, plate 3, and by Goodspeed and Clausen, plate 41, figure 1. The typical leaf and flower characters are well represented in plate 80 herewith.

In stature *virginica* belongs to the moderate pyramidal group of *Tabacum* varieties. It is conspicuously taller than *calycina,* the cen-

tral axis reaching a height of 150 to 175 cm. While strong laterals develop they do not originate at the base of the plant as in *calycina,* and they do not overtop the central axis. These characteristics together with the broad spreading basal leaves give the plant its pyramidal or conical shape. The leaves are very close in general shape to those of *calycina,* but they taper less abruptly to either end. The apex is prolonged into a fairly long point curved to one side, and the base is expanded into two broad, partially clasping auricles.

The inflorescence consists of a more ample panicle than that of *calycina.* The flowers are light pink in color, identical in this respect with those of *angustifolia.* The tube and infundibulum are narrow, gradually increasing in diameter from below in a funnel-shaped fashion. The corolla lobes are broad at the base, but have long, slender incurved points. Capsule and calyx present no very characteristic features, although the calyx is persistent in contrast to the deciduous calyx of *calycina.*

It will be seen from the foregoing descriptions that there are a number of distinct character contrasts between *calycina* and *virginica,* a brief note of which may well be made at this point. In flower color, red of *calycina* is contrasted with light pink of *virginica,* the same contrast which existed in the *angustifolia-macrophylla* series. Similarly the split hose-in-hose flower of *calycina* is contrasted with the normal one of *virginica;* low stature with tall; and a less decided contrast in leaf shape exists, depending upon the presence or absence of auricles at the base of the leaf.

2. F_1 OF THE CALYCINA–VIRGINICA SERIES

In the season of 1910, 55 plants of $10F_1H_{18}$ and 58 of $10F_1H_{20}$ were grown. In the season of 1911, 10 plants were grown of each of $11F_1H_{18}$ and $11F_1H_{20}$.

Like other hybrids which have been grown, these populations were uniform and equivalent throughout. It was thought that $10F_1H_{20}$ showed a more distinctly pronounced trace of *calycina* characters than did $10F_1H_{18}$, but the populations of the same seed grown in 1911 showed no appreciable difference in this respect.

In general appearance the F_1 plants resembled *virginica* more than they did *calycina.* The plants were somewhat shorter than *virginica,* running up to 135 cm. In these plants it was noted that some of the laterals overtopped the central axis as they do in *calycina.* The inflorescence was in general of the ample type characteristic of *calycina.*

The flower color was a deep pink intermediate between the two parents. The flower shape was normal throughout save that on every plant there was a small percentage of calyces with one or more pink and somewhat broadened calyx tips, or with a streak of white on one side. Sometimes these partially petaloid calyces were partly decid-uous. In shape of flower the hybrid closely resembled *virginica* except that the corolla lobes were longer and more decidedly mucronate. Calyx and capsule were almost identical with those of *virginica*, the calyx being typically persistent and accrescent. The leaves were some-what broader proportionately than those of *virginica*, but they pos-sessed the pronounced auricles of that parent. The usual gradation in leaf shape on each plant from the broad basal leaves to the linear bracts of the inflorescence was in evidence.

The main features of H_{18} and H_{20} are well illustrated in the draw-ings of $10F_1H_{18}P_5$ shown in plate 81. The general habit and charac-ters are illustrated in the photograph of $10F_1H_{18}P_{54}$ which is repro-duced in plate 83, figure 1.

3. F_2 OF THE CALYCINA–VIRGINICA SERIES

In the season of 1911 four F_2 families of the *calycina-virginica* series were grown, viz., $11F_2H_{18}P_{25}$, $11F_2H_{18}P_{49}$, $11F_2H_{20}P_7$, and $11F_2H_{20}P_{26}$.

As in the *angustifolia-macrophylla* series, the segregation exhibited in those four families, comprising 97 plants, was nothing short of be-wildering, and in most cases an intergrading series of forms connected one character expression with another. However, an attempt was made to classify the plants into categories suggested by the four pairs of character contrasts existing between the parents. The results of this classification are given in table 8.

TABLE 8

NUMERICAL DATA FROM F_2 POPULATIONS OF THE *CALYCINA-VIRGINICA* SERIES.

Garden Numbers	Corolla color			Corolla shape			Stature			Leaf width	
	red	pink	light pink	hose-in-hose	partialhose-in-hose	normal	short	medium	tall	broad	narrow
$11F_2H_{18}P_{25}$	6	11	6	6	8	9	17	2	5	17	7
$11F_2H_{18}P_{49}$	7	14	4	12	4	9	19	3	3	14	11
$11F_2H_{20}P_7$	5	9	11	7	8	10	11	6	8	21	4
$11F_2H_{20}P_{26}$	5	10	8	10	6	7	12	5	6	15	8
Totals	23	44	29	35	26	35	59	16	22	67	30

In this cross, corolla color behaved in exactly the same manner as it did in the *angustifolia-macrophylla* series. The same remarks as to sharpness of segregation apply here as in that series. Red was nearly always readily distinguishable, but pink and light pink formed a more or less completely intergrading series. Taking the results in this way, we obtain 23 red; 73 pink and light pink, which is substantially in accord with Mendelian expectations.

TABLE 9

F₂ SEGREGATION IN *CALYCINA-VIRGINICA* SERIES.

Garden numbers	Pink normal	Pink hose-in-hose	Red normal	Red hose-in-hose
$16F_2H_{18}P_{25}$	24	14	8	3
$16F_2H_{18}P_{49}$	25	8	14	3
$16F_2H_{20}P_7$	29	6	10	4
$16F_2H_{20}P_{38}$	30	8	11	1
Totals	108	36	43	11
Expected	112	37	37	12

With respect to corolla form some difficulty was encountered because the expression of the hose-in-hose character in the segregants did not seem to be so extreme as it was in the parent, and a large number of the plants showed slight traces of it, but sometimes in a more pronounced form than in the F₁ hybrids. Accordingly the classification of corolla form in table 8 is not a wholly satisfactory one.

The classification for stature and leaf width is subject to similar remarks as to its definiteness. Here there was also a more or less completely intergrading series of forms and no accurate measurements were taken. However, there is no doubt that there was segregation with respect to these characters, and a range of forms was obtained which completely bridged the gap between the parents. The behavior of these characters is to be considered in the light of their segregation in subsequent generations.

In 1916 four additional F₂ populations of the *calycina-virginica* series were grown in order to reëxamine populations for the segregation of normal versus hose-in-hose flowers, and red versus pink flower color. The method of classifying the flowers was that used in studies of 1916 populations previously mentioned. The results of these studies are given in table 9.

In the segregation the same grading as before of the pinks into two intergrading classes in the proportion of approximately 2 intermediate pink : 1 light pink was observed, but it was even more difficult to draw a line between light pink and intermediate pink because of the effect of the hose-in-hose conditon on flower color expression in those plants which bore teratological flowers. In the matter of segregation into normal and hose-in-hose flowers, some difficulty was experienced because some otherwise normal flowering plants bore some flowers which showed a tendency for the calyx to become petaloid, and others bore flowers which showed a very slight hose-in-hose tendency. A correspondingly slight hose-in-hose tendency is also present in F_1 plants. These plants were classified as normal. Here again it can be seen that the segregation ratios of 144 pink : 54 red and 151 normal : 47 hose-in-hose are in substantial agreement with Mendelian expectation for contrasts in a single pair of allelomorphs in each case. Moreover, the dihybrid ratio is substantially in agreement with that expected for independent segregation of the members of these two pairs of allelomorphs.

4. F_3 AND SUBSEQUENT GENERATIONS OF THE CALYCINA–VIRGINICA SERIES

In 1912 twelve F_3 families of H_{18} and five of H_{20} were grown. They will be grouped for consideration according to the characters which

TABLE 10

F_3 BEHAVIOR OF RED SEGREGANTS.

Garden numbers	Red
$12F_3H_{18}P_{25}P_{11}$	25
$12F_3H_{18}P_{25}P_{19}$	25
$12F_3H_{18}P_{49}P_{12}$	25
$12F_3H_{18}P_{49}P_{24}$	25
$12F_3H_{20}P_{26}P_{25}$	25

the F_2 parent exhibited. In table 10 the data with respect to the behavior of F_3 populations from red flowering F_2 plants are collected. Five such populations gave nothing but red flowering plants, indicating clearly that red segregants breed true. In table 11 the data from pink flowering plants are similarly collected. The reader will not fail to notice that some pink flowering selections were not heterozygous for

red. This bears out our statements as to the difficulty of classifying pink and light pink. In the seven populations which produced red flowering plants 38 plants had red flowers and 134 pink or pinkish flowers; again in substantial agreement with the behavior of flower color in the *angustifolia-macrophylla* series. The behavior of segregants classified as light pink is shown in table 12. Of the four populations from which data were gathered only one bred true to light

TABLE 11

F_3 BEHAVIOR OF PINK SEGREGANTS.

Garden numbers	Red	Pink	Light pink
$12F_3H_{18}P_{25}P_7$	5	16	4
$12F_3H_{18}P_{25}P_{21}$	....	21	4
$12F_3H_{18}P_{25}P_{25}$	6	19	....
$12F_3H_{18}P_{49}P_9$	4	20	....
$12F_3H_{18}P_{49}P_{10}$	11	13	....
$12F_3H_{18}P_{49}P_{25}$	4	16	5
$12F_3H_{20}P_{26}P_6$	4	20	...
$12F_3H_{20}P_{26}P_{11}$	4	21	....
Totals for segregating populations	38	134	

TABLE 12

F_3 BEHAVIOR OF LIGHT PINK SEGREGANTS.

Garden numbers	Red	Pink	Light pink
$12F_3H_{18}P_{25}P_{24}$	....	....	23
$12F_3H_{18}P_{49}P_{22}$	8	12	5
$12F_3H_{20}P_7P_9$	6	15	4
$12F_3H_{20}P_{26}P_5$	....	25	...

pink, one of the others bred true for pink, possibly a slightly darker shade than true light pink, and two segregated for all three colors; they must therefore have been pink heterozygotes.

In F_4 two populations each of H_{18} and of H_{20} were grown. Population $13F_4H_{18}P_{25}P_{11}P_9$ from an F_3 population breeding true for red gave in F_4 100 plants all red flowering. Population $13F_4H_{18}P_{25}P_{11}P_{13}$ from the same F_3 population gave 97 plants all red flowering like *calycina*. Population $13F_4H_{20}P_{26}P_5P_8$, which bred true for pink in F_3, gave in F_4 96 plants, all pink flowering. These three populations were grown to F_7 without showing further evident segregation. The

pink of the pink flowering derivative was at first considered somewhat darker in shade than the light pink of *virginica,* but this line also showed the hose-in-hose flower character, which sometimes makes it difficult to determine flower color accurately. In later generations of this line its color was noted as equivalent to the light pink of *virginica.*

TABLE 13

F_3 BEHAVIOR OF HOSE-IN-HOSE SEGREGANTS.

Garden numbers	Hose-in-hose
$12F_3H_{18}P_{25}P_{21}$	25
*$12F_3H_{18}P_{25}P_{24}$	23
$12F_3H_{18}P_{49}P_9$	24
*$12F_3H_{18}P_{49}P_{12}$	25
$12F_3H_{18}P_{49}P_{24}$	25
$12F_3H_{20}P_{26}P_6$	24
$12F_3H_{20}P_{26}P_{11}$	25

* Apparently not so extreme as *calycina.*

TABLE 14

F_3 BEHAVIOR OF NORMAL SEGREGANTS.

Garden numbers	Hose-in-hose	Partial	Normal
$12F_3H_{18}P_{25}P_7$	9	....	16
$12F_3H_{18}P_{25}P_{11}$	4	4	17
$12F_3H_{18}P_{25}P_{19}$	5	20	
$12F_3H_{18}P_{25}P_{25}$	3	....	22
*$12F_3H_{18}P_{49}P_{10}$	....	4	20
$12F_3H_{18}P_{49}P_{22}$	....	....	25
$12F_3H_{18}P_{49}P_{25}$	4	21	
$12F_3H_{20}P_7P_9$	5	20	....
$12F_3H_{20}P_{26}P_5$	7	3	15
$12F_3H_{20}P_{26}P_{25}$	10	....	15
Totals	47	153	

Taking up corolla form next, we may deal with the different populations in the same manner as was done in the case of flower color. F_3 populations from F_2 hose-in-hose segregants are recorded in table 13. Seven populations were grown, all of which bred true to the hose-in-hose character, although curiously enough two populations, $12F_3H_{18}P_{25}P_{24}$ and $12F_3H_{18}P_{49}P_{12}$ did not appear to exhibit so extreme character expressions as *calycina.*

Only one partially hose-in-hose plant was grown in F_3. For the sake of economy of space it is included in table 14, where it is marked

with an asterisk. Strangely enough, it was one of the two in the table which did not throw hose-in-hose flowers. The other normal selections all threw hose-in-hose flowering plants in the proportion of about 3 normal to 1 hose-in-hose.

In subsequent generations only the three families which were previously considered under flower color were grown. Normal flower selections from $12F_3H_{18}P_{25}P_{11}$ gave two populations, one of 100 and one of 97 plants. The plants all bore normal flowers. In $13F_4H_{18}P_{25}P_{11}P_{13}$ it was noted that some flowers were split, but there was not even a suggestion of approach to the true hose-in-hose condition. The other population $13F_4H_{20}P_{26}P_5P_8$ was from a hose-in-hose selection in the

TABLE 15

F_3 BEHAVIOR OF TALL SEGREGANTS.

Garden numbers	Tall	Short
$12F_3H_{18}P_{25}P_7$	19	6
$12F_3H_{18}P_{25}P_{24}$	1	24
$12F_3H_{20}P_7P_9$	6	19

TABLE 16

F_3 BEHAVIOR OF MEDIUM SEGREGANTS.

Garden numbers	Tall	Short
$12F_3H_{18}P_{49}P_{12}$	....	23
$12F_3H_{18}P_{49}P_{22}$	1	24

corresponding F_3 population. Ninety-four plants were grown to maturity, all of which were strictly hose-in-hose. In subsequent generations these three populations bred true to type save for the sporadic appearance of hose-in-hose flowers on plants which otherwise bore nothing but normal flowers. This, however, is not an unusual phenomenon even in pure line cultures of normal flowering varieties of *Tabacum,* and it is extremely doubtful whether the hybrid derivation of these plants had anything to do with the production of occasional split flowers.

As respects height of plants the F_3 data are given in tables 15, 16, and 17, which give the behavior of tall, medium, and short F_2 segregants respectively. The behavior here is not very convincing. Probably the difficulty in judging the character and the influence of variation in soil condition had something to do with it.

In the subsequent generations the behavior was, however, more definite. $13F_4H_{18}P_{25}P_{11}P_9$ was grown from a tall F_3 plant. No definite notes were taken as to height, but the population was noted as varying. In F_5 and subsequent generations the line bred true to tall. $13F_4H_{15}P_{25}P_{11}P_{13}$ was grown from a short F_3 plant. The ninety-seven plants were all of low stature and in subsequent generations the line bred true for low stature. $13F_4H_{20}P_{26}P_5P_8$ was grown from a tall F_3 plant. Ninety-four plants, although variable in height, all belonged in the tall class and in subsequent generations the line bred true for tall stature. Nothing but a careful biometrical study under

TABLE 17

F₃ BEHAVIOR OF SHORT SEGREGANTS.

Garden numbers	Tall	Short
$12F_3H_{18}P_{25}P_{11}$	10	15
$12F_3H_{18}P_{25}P_{19}$	16	9
$12F_3H_{18}P_{25}P_{21}$	....	25
$12F_3H_{18}P_{25}P_{25}$	5	20
$12F_3H_{18}P_{49}P_9$	1	24
$12F_3H_{18}P_{49}P_{10}$	....	24
$12F_3H_{18}P_{49}P_{24}$	7	18
$12F_3H_{18}P_{49}P_{25}$	9	16
$12F_3H_{20}P_{26}P_5$	8	16
$12F_3H_{20}P_{26}P_6$	5	19
$12F_3H_{20}P_{26}P_{11}$	....	25
Totals	68	129

well controlled cultural conditions, however, would yield results capable of strict Mendelian analysis. However, it can be said that none of the results here recorded preclude the possibility of such an analysis, although it evidently can not be done in any simple qualitative manner.

As respects leaf width it was found impossible to make even such a rough classification as was attempted in the case of stature. Here again nothing short of a strict biometrical analysis would furnish the basis for a Mendelian formulation.

As has been indicated above, three separate lines of this series were carried out to the seventh hybrid generation. Of these, one was a recombination of characters from both parents exhibiting the tall stature and normal flower of *virginica* with the red flower color of *calycina*. One exhibited a stature intermediate between that of *calycina* and *virginica* in combination with the normal flower shape of

virginica and the red flower color of *calycina*. The third had the tall stature of *virginica* and red flower color, in association with the hose-in-hose flower form of *calycina*. These three lines apparently bred true for all their characters.

5. DISCUSSION OF RESULTS OF THE CALYCINA–VIRGINICA SERIES

No extended discussion of results is indicated in connection with the *calycina-virginica* series of hybrids because particular attention was given to so few characters. Just as in the case of *angustifolia-macrophylla,* so in this series of hybrids the character differences proved to depend upon complex genotypic differences. Apparently the flower color contrast in these two varieties was the same as that in the *angustifolia-macrophylla* series, and the same relations with respect to dominance and segregation were found to hold for it. Without doubt we are dealing here with the **Rr** pair of allelomorphs as in the previous instance. The demonstration of the simple factor relations in the inheritance of the split hose-in-hose form of flower adds to our series another pair of allelomorphs which we may call **Cc** (calycine). In this case the dominance of normal over split hose-in-hose appears to be nearly, if not quite, complete. The sporadic appearance of split hose-in-hose flowers on otherwise normal plants does not even seem to be clearly associated with the heterozygous genotype, **Cc**. The data for height are not of sufficient accuracy or extent to warrant an attempt at Mendelian formulation. It was again found possible very easily to shuffle and recombine the characters occurring in the parent varieties and to establish recombination derivatives in pure lines.

V. ALBA–MACROPHYLLA SERIES

1. PARENTS OF THE ALBA–MACROPHYLLA SERIES

Alba, which is one of the parents of the *alba-macrophylla* series, is the ''White'' tobacco, U. C. B. G. 30/06, described by Setchell. It is one of the taller forms of *Tabacum,* ranging in height from 165 to 220 cm. Typically *alba* is unbranched below; above, it has flowering branches corymbosely arranged in succession from above downward. The leaves are sessile, more ample, more rugose, and more velvety than those of *macrophylla*. They are narrowed suddenly above the expanded, somewhat auricled and partially clasping base. The leaves

resemble those of *macrophylla* in shape but differ from them particularly in the basal portion. The corollas are white with a yellowish tinge; but in shape, size, and general proportions they are very similar to those of *macrophylla*. Line drawings of typical features of *alba* are reproduced in plate 82. The reproductions of photographs of the leaf of *alba* and of the F_1 hybrid of the *alba-macrophylla* series are shown in plate 84.

Macrophylla, U. C. B. G. 22/07, has been described above.

In these two varieties there are definite character contrasts in color of flowers, *macrophylla* being red and *alba* white; and in stature, *macrophylla* being low of stature and *alba* distinctly taller. Other contrasts also exist, although they are not so definite, in the style of branching and in the shape and texture of the leaves. Like those which have been considered above, this is a hybrid series in which the contrasts between the parent forms are of a distinctly complex character.

2. F_1 OF THE ALBA–MACROPHYLLA SERIES

The crosses between *alba* and *macropyhlla* were made in July, 1909. The cross was successful in both directions, and seed was secured from *alba*♀ × *macrophylla*♂, which was given the number H_{23}, and from the reciprocal which was given the number H_{24}.

When mature the F_1 plants were tall, 100 to 200 cm., averaging 130 to 160 cm. Habit and leaf shape were in general those of *alba*. The corolla was deep pink of about the same shade as that of the F_1 of the *angustifolia-macrophylla* series. The variation in height in these populations possibly indicates a lack of constancy in the *alba* parent in this respect. In plate 83, figure 2, is shown an F_1 plant of $10F_1H_{24}$.

3. F_2 OF THE ALBA–MACROPHYLLA SERIES

In 1911 four F_2 populations were grown, viz., 25 plants each of $11F_2H_{23}P_{13}$, $11F_2H_{23}P_{31}$, and $11F_2H_{24}P_6$, and 23 plants of $11F_2H_{24}P_{34}$. The four populations, although small, proved to be equivalent in every respect. The type of segregation was very complex. That of differences in types of leaves, especially, presented such a series of intergradations as to defy any definite classification. Likewise in height, there was a continuous series of forms from the tallest to the shortest. A rough classification was, however, made for purposes of reference into tall, medium, and short. An excellent illustration of the segregation for this character is shown in plate 85, figure 1, which shows

two adjacent plants of $11F_2H_{24}P_{34}$, one tall and of the general habit of *alba*, and the other short and of the general habit of *macrophylla*. The classification for height is given in table 18. Obviously no satisfactory Mendelian formulation can be deduced from these data.

As regards flower color, however, the classification is more definite. Four more or less distinct shades were distinguishable, viz., red, pink, light pink, and white. The pink and light pink shades merged into each other, consequently they have not been separately recorded in table 18. Bearing in mind the previous behavior of red and pink, as shown in the *angustifolia-macrophylla* and *calycina-virginica* series,

TABLE 18

F2 SEGREGATION IN THE *ALBA-MACROPHYLLA* SERIES.

Garden numbers	Stature			Flower Color		
	tall	medium	short	red	pink	white
$11F_2H_{23}P_{13}$	11	6	8	3	14	8
$11F_2H_{23}P_{31}$	13	6	6	4	12	9
$11F_2H_{24}P_6$	7	10	8	3	15	7
$11F_2H_{24}P_{34}$	6	8	9	6	13	3
Totals	37	39	31	16	54	27

it would appear that we are here dealing with dihybrid populations in which a pair of allelomorphs for color versus white is concerned in addition to that pair upon which the contrast of pink versus red was found to depend. The pair of allelomorphs for the pink versus red contrast has been represented by **R** and **r**, respectively. If we represent the contrast of color versus white by **W** and **w**, respectively, the two parents in this series would possess the following genotypes:

$$Alba \qquad = \textbf{RR}\text{ww}$$
$$Macrophylla = \text{rr}\textbf{WW}$$

The light pinks of the previous series would then be **RRWW**, and the factor **R** might be regarded as a dominant diluter. According to this formulation, F_1 of the *alba-macrophylla* series would be **RrWw**, pink, and F_2 should segregate in the ratio 3 red : 9 pink : 4 white. The expected result in the classification of ninety-seven plants in whole numbers is 18 red : 55 pink : 24 white. Agreement is thus fairly close.

A check on the results above noted for the 1911 sowings of the F_2 population was made by growing in 1916, five additional F_2 popula-

tions of the same series, viz., $16F_2H_{23}P_5$; $16F_2H_{23}P_{32}$; $16F_2H_{23}P_{34}$; $16F_2H_{24}P_{28}$; and $16F_2H_{24}P_{33}$. The segregation in the resulting populations is recorded in table 19.

The method of studying these flowers was the more accurate one previously described in connection with later generations of the *angustifolia-macrophylla* series. In the classification of flowers it was noted that reds and whites were sharply distinguishable from pinks. The pinks were of many different shades; some very light, others relatively dark, corresponding to the range obtained in the *angustifolia-macrophylla* series. However, in these populations the range of vari-

TABLE 19

F₂ SEGREGATION IN 1916 SOWINGS OF *ALBA-MACROPHYLLA SERIES.*

Garden numbers	Red	Pink	White	Totals
$16F_2H_{23}P_5$	11	18	11	40
$16F_2H_{23}P_{32}$	12	19	11	42
$16F_2H_{23}P_{34}$	8	24	9	41
$16F_2H_{24}P_{28}$	5	34	11	50
$16F_2H_{24}P_{33}$	7	29	14	50
Observed	43	124	56	223
Expected	42	125	56	223

ation of pink appeared to be greater and the intergradations more gradual than in that series. In the whites there was also evidence of differentiation into classes depending upon the amount of yellow or creaminess in the flowers. Some of the whites appeared to belong to a clear white albino class, but most of them had a distinctly creamy tinge. The observed segregation in these populations was in almost exact agreement with the formulation advanced above.

4. F₃ AND SUBSEQUENT GENERATIONS OF THE ALBA–MACROPHYLLA SERIES

In table 20 we have summarized the behavior of the F_3 populations as respects color of flowers and height of plant. Of the five red F_2 plants from which F_3 populations were grown, three proved to be homozygous for red and one proved to be a heterozygote of the genetic constitution **rrWw**. Of this latter selection two sowings were made, one in 1912 and another in 1913. The combined results from these two sowings, 35 red: 14 white, are in fair agreement with Mendelian

expectation. The other population exhibited an anomalous type of segregation, and gave 2 red : 23 white. It is unfortunate that this line was not investigated further, but the results probably are due to an experimental error.

TABLE 20

F₃ SOWINGS OF THE *ALBA-MACROPHYLLA* SERIES.

F₂ Phenotypes		Garden Numbers	Flower Color			Stature	
Flower Color	Stature		Red	pink	white	short	tall (or medium)
Red	Tall (or M)	$12F_3H_{23}P_{13}P_3$	19	....	6	25	....
	Short	$12F_3H_{23}P_{31}P_1$	22	....	....	22	....
	Sh't (or M)	$12F_3H_{23}P_{31}P_{17}$	25	....	....	4	21
	Tall	$12F_3H_{23}P_{31}P_{22}$	24	....	....	4	20
	Tall	$12F_3H_{23}P_{31}P_{25}$	2	....	23	1	24
	Tall	$16F_3H_{23}P_{13}P_3$	16	....	8	....	....
	Tall	$12F_3H_{23}P_{13}P_{13}$	2	14	6	8	10
	Medium	$12F_3H_{23}P_{13}P_{25}$	6	12	7	22	....
	Tall	$12F_3H_{23}P_{31}P_7$	6	14	5	....	25
	Tall	$12F_3H_{23}P_{31}P_{19}$	10	9	4	....	23
Pink	Medium	$12F_3H_{23}P_{31}P_{20}$	6	12	5	4	19
	Tall	$12F_3H_{24}P_6P_5$	3	22	....	....	25
	Short	$12F_3H_{24}P_{34}P_{18}$	5	13	7	24	1
	Tall	$12F_3H_{24}P_{34}P_{20}$	....	20	5	....	25
	Tall	$16F_3H_{24}P_{34}P_{20}$	....	13	4	....	....
	Tall	$12F_3H_{23}P_{13}P_{14}$	....	....	25	....	25
	Tall	$12F_3H_{23}P_{13}P_{15}$	....	....	25	....	25
	Short	$12F_3H_{23}P_{13}P_{24}$	....	....	25	25	....
White	Tall	$12F_3H_{24}P_6P_2$	....	....	24	....	24
	Short	$12F_3H_{24}P_6P_3$	....	....	25	4	21
	Tall	$12F_3H_{24}P_6P_4$	....	....	23	....	23
	Short	$12F_3H_{24}P_{34}P_{23}$	....	1	23	25	....
	Short	$13F_3H_{23}P_{13}P_{24}$	....	....	10	10	....
	Short	$13F_3H_{24}P_{34}P_{23}$	....	....	10	10	....

Eight families of F₃ plants were grown from pink F₂'s. Of these F₂ plants six proved to belong to the **RrWw** genotype. The totals from these six populations, viz., 35 red : 74 pink : 34 white, are in fair agreement with the dihybrid ratio 3 red : 9 pink : 4 white. One of the other populations gave 3 red : 22 pink. It was probably the result of sowing seed from an F₂ plant of the genetic constitution **RrWW** which should give 3 pink : 1 red. The observed segregation ratio is not good, but the numbers are small. Two sowings of $F_3H_{24}P_{34}P_{20}$ gave totals of 33 pink : 9 white. The F₂ plants in this case must have been of the genetic constitution **RRWw**; in which case expectation would be 3 pink : 1 white. No selection was observed to breed true

for pink in F_2. This, however, is not inexplicable, for only one in nine among the F_2 pinks should belong to the **RRWW** genotype.

Sowings were made from seven white F_2 plants. Among 190 plants so produced there was one pink flowering individual. It surely represents some kind of experimental error. We may say, therefore, that for flower color the formulation advanced to account for the F_2 segregation ratio, also accounts for the behavior observed in the various F_3 populations.

We have reported the data on height in table 20, largely in order to show that this character, although obviously dependent on factor differences, is so complex as not to permit of a simple qualitative treatment. Thirteen F_3 sowings from tall F_2 plants gave ten populations showing only tall plants. Two of the remaining populations showed segregation into 31 tall (and medium) : 13 short. One population consisted entirely of short plants. The classification of the F_2 parent of this plant as ''tall'' was noted as doubtful at the time, the note ''or medium'' being appended. Two-populations were grown from F_2 plants of medium height. One of these populations was uniformly of low stature; the other showed segregation into 19 tall (and medium) : 4 short. Six populations were grown from F_2 parents, four of which apparently bred true for low stature, the other two showed segregation into tall (and medium) or short in the ratio 42 : 8. It is interesting to note that at the time of classification the parents of these two later populations were classified as short (or medium), indicating a doubt as to proper classification. More definite data will be necessary before a satisfactory formulation of these height differences can be made, but certain of our results seem to indicate that there is one allelomorphic pair which has a rather marked effect on stature, and that there are other subsidiary pairs of factors which have less marked effects.

Only one line in this *alba-macrophylla* series was carried out to subsequent generations to demonstrate the possibility of fixing character complexes from a hybrid. It was a low stature white flowering line. In F_4, 100 plants of $13F_4H_{24}P_{34}P_{23}P_2$ bred true to low stature and white flower color. The population was uniform, the plants exhibited the general habit of *macrophylla* rather than that of *alba;* and the leaves were the same shape as those of *macrophylla*, but they were slightly rugose, although not so much so as those of *alba*. In F_5, two populations of 25 plants each were grown, viz., $14F_5H_{24}P_{34}P_{23}P_2P_{47}$ and $14F_5H_{24}P_{34}P_{23}P_2P_{83}$. No differences were detectable between these two populations, and the characters exhibited were those we have noted

for F_4. In F_6 10 plants each of $15F_6H_{24}P_{34}P_{23}P_2P_{47}P_5$ and $15F_6H_{24}$ $P_{34}P_{23}P_2P_{83}P_{12}$ and in F_7 10 plants each of $16F_7H_{24}P_{34}P_{23}P_2P_{83}P_{12}P_8$ and $16F_7H_{24}P_{34}P_{23}P_2P_{47}P_5P_8$ were grown. In both cases the parallel populations were equivalent and the characters exhibited and described in F_4 remained constant. Plate 85, figure 2, is a good illustration of the type of this family as fixed. A photograph of the original F_2 plant, from which the family descended, is reproduced in plate 85, figure 1. It will be noted that the derivative represents a fixation of the characters of the original F_2 selection, and that no important segregation occurred in it either in F_3 or in subsequent generations.

5. DISCUSSION OF RESULTS OF THE ALBA–MACROPHYLLA SERIES

Here again, as in the *calycina-virginica* series, no extended discussion of results is necessary. Obviously the differences separating the two varieties are of a complex nature genetically as in the two previous cases. The series demonstrates the existence of another pair of allelomorphs for flower color in this group of *Tabacum* varieties, viz., **Ww,** and the part played by it in the production of both red and light pink flower color has been determined. The height contrast again proves to be too complex for qualitative Mendelian formulation. As in the previous cases, the establishment of stable recombination derivatives proved to be a simple task.

VI. GENERAL CONCLUSIONS

We shall limit the discussion of these results to three main topics upon which these investigations seem to have thrown some light: (1) the origin and interrelationships of varieties of *Tabacum;* (2) the methodology of Mendelian analysis in *Tabacum;* and (3) Mendelian heredity in *Tabacum.*

1. ORIGIN AND INTERRELATIONSHIPS OF VARIETIES OF TABACUM

As a result of extensive studies of a considerable assemblage of *Tabacum* varieties, Comes (1905) came to the conclusion that the species *Tabacum* could be subdivided into six fundamental varieties:

  a. var. *fructicosa* Hook.
  b. var. *lancifolia* (W.) Comes.
  c. var. *virginica* (Agdh.) Comes.
  d. var. *brasiliensis* Comes.
  e. var. *havanensis* (Lag.) Comes.
  f. var. *macrophylla* Shrank.

Inasmuch as practically every *Tabacum* variety shows combinations of characters of two or more of these fundamental varieties, Comes assumed them to have been derived mostly through hybridization between the fundamental varieties, and he proceeded from purely morphological studies to classify the different commercial varieties on the basis of their supposed hybrid derivation. Anastasia (1906), who has criticized this scheme of Comes very severely, reduced the number of fundamental varieties to four, striking out *fructicosa* and *lancifolia* from Comes' list, and substituting *purpurea* for *macrophylla*. Although disagreeing as to the fundamental varieties, Comes and Anastasia seem to agree in referring existing varieties to derivation, mostly through hybridization, from a relatively small number of fundamental varieties.

The Howards (1912) object to the mode of classification of Comes and Anastasia, and point out as a result of their studies of types of Indian tobaccos that no attempt at classification based on derivation can be considered seriously unless supported by actual experimental studies. In her later paper in particular Miss Howard (1913) shows that segregation products may be obtained through hybridization which transcend the limits set by the parents. The Howards propose a scheme of classification based primarily upon leaf and habit characters, and they adopted this morphological system purely as a provisional means for facilitating identification and reference among the numerous forms of Indian tobaccos.

Our results agree with those stated by the Howards, and we raise the same objection to schemes of classification such as Comes and Anastasia have advocated. Any scheme of classification based on morphological considerations alone cannot well meet with the approval of geneticists, for it does not take into account genotypic differences which exist among forms of similar morphological appearance. Thus it is possible, as Miss Howard points out, by crossing different members of a given group to obtain segregation products which belong in an entirely different morphological group in the scheme of classification. In particular she points out that "petiolate" forms have been produced as segregation products from two "sessile" parents, yet "petiolate" and "sessile" have been used as primary indexes for classification of tobaccos into groups.

The difficulty from the genetic point of view with any classification of *Tabacum* varieties is the same as that which is met with in the classification of varieties of other polymorphic species. Taking the species as a whole and viewing the entire assemblage of its varieties,

there is evidently in *Tabacum,* to those who accept current interpretations of heredity, a series of allelomorphic contrasts, the number of which cannot even be guessed, but which need not perhaps be more numerous or striking than those which have been discovered in *Drosophila.* But whereas in *Drosophila* the factors have been kept in stocks involving for the most part single factor differences from a common wild type, in *Tabacum,* and in other cultivated crop plants such as barley, maize, oats, rice, wheat, etc., these factor differences have been shuffled about through long periods of cultivation until existing varieties are no longer related clearly to a common form or to each other. In some instances in such groups certain factor differences have a more striking visible effect than in others. In such instances we have an obvious mode of classification based not upon number of factor differences so much as upon the striking character differences which arise from certain factor contrasts. Thus in barley we have the classification of varieties advocated by Harlan (1918) based upon recognition of a number of major morphological distinctions, some of which at least have been clearly analyzed in Mendelian fashion; and the same principle has been recognized in the classification of varieties of maize, where it has led to the absurdity of erection of a heterozygous form, podded maize (*vide* Collins), as one of the primary group distinctions. In some instances, doubtless, the sorting of factors may give rise to certain recombinations which are more favorable to life processes than others, as Muller has pointed out in another connection, and such genotypes may act as centers around which groups of varieties may be built up, thus giving rise to more or less obvious grouping of varieties. The attempt to base a system of classification upon reference to certain fundamental types does not, however, promise much simplification of the difficulty; moreover, such an attempt rests upon the rather naive assumption that it is unnecessary to account for the fundamental types.

From a genetic standpoint, therefore, it would appear that in attacking the problem of classification and interrelations of varieties in a polymorphic species the major premise should be a recognition of the fundamental equivalence of every homozygous genotype. Starting from this premise a system of dichotomy beginning with those factor contrasts which produce the most striking, visible effects and proceeding to those of lesser effect might be set in operation. Such a system obviously would in certain cases separate some similar varieties into separate groups, and would lead to recognition of group differences without obvious morphological distinctions, but the system

would have a real significance, and the relationships indicated by it would be fundamental ones. It is, however, necessary to have a much more extensive knowledge of Mendelian heredity in *Tabacum* than we have at present before such a system can be formulated.

2. METHODOLOGY OF MENDELIAN ANALYSIS IN TABACUM

From the Mendelian side there are certain obvious facts associated with *Tabacum* as a species. In the first place, as we have stated before, the species is highly polymorphic. A large and striking assemblage of varieties exists, the most extreme of which hybridize readily and give fully fertile hybrids and full fertility in their derivatives. A few teratological forms are known in which fertility is somewhat reduced, but the above generalization does not far overstate the facts. The species is, moreover, so highly polymorphic that with respect to any given character a representative collection of varieties may be arranged in a series connecting the most extreme expressions of that character by imperceptible steps. Thus in flower color we have represented in the collection of varieties of the University of California Botanical Garden dark red, red, light pink, pinkish, and white, and descriptions occur in the literature which indicate the existence of further shades of red connecting these. Now flower color is a rather definite character, comparatively speaking, for it appears to be little affected by ordinary environmental conditions. In many polymorphic forms, such for example as maize, there are a large number of such definite characters, and as a consequence studies of inheritance in these forms have resulted in definite Mendelian analysis of many character differences. But in *Tabacum* unfortunately most of the characters involve quantitative elements, and these with few exceptions depend so largely for their particular expression upon environmental conditions that it becomes a difficult matter in a segregating population to distinguish between those differences which are inherent and referable to the genotype and those which have come about through the action of extrinsic forces. And yet our assemblage of tobacco varieties indicates clearly that there are genotypes which give rise to all possible expressions in these characters. Here we find the reason for the present backward state of knowledge of inheritance in *Tabacum*, for while there have been numerous investigations which indicate clearly that the Mendelian mode of transmission may be followed in all these character differences, yet there are very few investigations which have resulted in the precise type of factor analysis characteristic

of investigations with other forms, specific mention of which is un-
necessary.

The general features of inheritance in *Tabacum* varietal crosses
are plain enough. The results of our investigations in this connection
agree throughout with the conclusions which Miss Howard drew from
her studies. When we are dealing with complex differences, the F_1
is commonly intermediate in character expression between the two
parents. Not only is this true as respects the F_1 plant as a whole but
it is also true for individual characters. The F_2 commonly consists
of a varied assemblage of forms covering the range between the two
parents, or even not uncommonly presenting products not included
in the range between the two parents. So many and of such variety
are the forms obtained that accurate classification is entirely out of
the question. But in F_3 and in subsequent generations segregation,
even for characters commonly regarded as quantitative, sometimes
occurs in distinct discontinuous classes in marked contrast to the inter-
grading series of forms obtained in F_2. This is shown particularly
well in our analysis of leaf base factors, for in this case we have been
able to adopt a qualitative mode of attack on one of the features which
contributes to leaf shape. If such an analysis proves successful in
one instance, there seems to be little reason why it should not be ex-
tended to others. There is, therefore, additional evidence in this suc-
cessful application of the mode of qualitative analysis to quantitative
characters in support of the oft repeated contentions of East (1913),
Hayes (1912), Hayes, East, and Beinhart (1913), Miss Howard
(1913), and others that fundamentally the same mode of inheritance
holds for quantitative characters in tobacco as for qualitative ones.
The distinction between the two classes of characters is purely an
artificial one erected for the purpose of convenience in formal treat-
ment, and at most depending merely upon an increase in complexity
of the factor relations involved and on the greater fluctuation of the
characters in response to environmental differences.

The question remains to be discussed whether semiquantitative
characters admit of a qualitative mode of analysis, and if so, how?
Miss Howard (1913) as a result of her extensive studies of inheritance
in Indian tobaccos concludes that the easiest way to determine the
principles underlying inheritance in these forms is to establish as
many extracted homozygous intermediate forms as possible. The estab-
lishment of such forms in themselves, however, is only a step in the
Mendelian analysis of the differences. Such forms are, as might have
been expected on theoretical grounds alone, less different from one

another and from the parents than the original parents are from each other. Moreover, our experiments show that as a result of simplification of the factor differences the derivative strains crossed with each other or with the parents give F_2 progenies which often exhibit clear-cut segregation in characters which showed intergrading series in the original F_2 population. In other populations, however, from crosses between derivatives, the populations still exhibit perplexing complexities which make classification difficult and uncertain. In such cases we could again resort to the method of establishing intermediate derivatives from them; but if the number of factors concerned in a given character is even moderately large, as is certainly the case with many of these quantitative characters, the number of genotypically different derivatives which may be secured becomes so great as to make the method impracticable.

Our experience indicates that the successful factor analysis of these quantitative character differences depends not only upon getting what Castle (1919) has called the residual heredity equivalent throughout the population, but also in establishing the proper kind of residuum which will most emphasize the character differences associated with the pair of factors or pairs of factors under investigation. The problem may be illustrated crudely by considering the pair of flower color factors **Rr**. If the residuum should contain **PP**, the effect of which is described below, segregation would give **PPRR**, **PPRr**, and **PPrr**. In character expressions these three different genotypes would doubtless all be of various shades of dark red, difficult or impossible of accurate separation. With such a residuum, therefore, it would be impossible to investigate satisfactorily inheritance in the factor pair **Rr**. But if we should substitute **pp** for **PP** in the residuum, the segregation products would be **ppRR** and **ppRr**, which would be pink, and **pprr**, which would be red. Here the segregation would be sharp and distinct, and there would be practically no difficulty in classification. How complex such interrelations can be has been shown most clearly by Bridges (1919) in his account of specific modifiers of eosin in *Drosophila*. As Bridges shows it would easily be possible to obtain populations of *Drosophila* defying classification, but by keeping the factors separate and studying their character effects with known residual genotypes, it has been possible to determine and locate the factors involved. Doubtless much of the extraordinary success of Mendelian analysis in *Drosophila* has been due to the fact that factor differences arose under conditions such that the residual genotype gave no difficulty; whereas in crop plants, the geneticist starts with

long established diverse types, evidently related to one another in fundamentally the same manner as are the various *Drosophila* mutants, but more complexly, and from these complex assemblages he must unravel the tangled skein of heredity.

There are, however, other and perhaps quicker ways of establishing a uniform and favorable residual heredity than that of securing and testing homozygous extractives, and these may be employed in certain special cases. Thus, if it be desired to study the relationship of the pair of factors **Ss** for the petioled versus sessile condition, it should be possible to proceed by crossing back the F_1 of *angustifolia* × *macrophylla,* for example, to *macrophylla,* selecting the petioled forms from the back cross for again crossing back to *macrophylla,* and continuing the process until clear-cut segregation was obtained. Such a mode of procedure should establish a residual genotype equivalent to that of *macrophylla* itself, and should thereby enable the student eventually to study the effect of substituting **SS** for **ss** in the *macrophylla* genotype. In tobaccos technical details make it particularly easy to adopt such a procedure, but it is useless to speculate further upon its results until it shall have been attempted.

3. MENDELIAN HEREDITY IN TABACUM

From the standpoint of factor analysis, we have demonstrated clearly in the foregoing pages, the existence of a number of distinct pairs of factors. Two of these affect flower color, one flower form, and three affect the character of the leaf base. The particular effects of the opposing members of these pairs of factors and the interrelations which they exhibit so far as these have been investigated have been set forth in the discussions which follow the description of each of the three series of hybrids. Although evidently many other factor differences were concerned in these studies, and remain for further investigation, the results which we have described make a beginning toward a more accurate knowledge of Mendelian heredity in *Tabacum.*

So far as our results furnish any data on the question, the six pairs of factors isolated exhibit no linkage relations. The data here are far from complete, but the results are in accordance with theory. According to White (1912), there are twenty-four pairs of chromosomes in *Nicotiana.* Assuming for the sake of discussion that each of these pairs of chromosomes bears a set of factors comparable in numbers to any other pair, then the chances of finding linkage when only six pairs of factors are studied is very slight. This large number

of pairs of chromosomes may account for the ease with which recombination pure lines were established. Even with a large number of factor differences, such as evidently distinguish these *Tabacum* varieties, the chances are slight with so many pairs of chromosomes that linkage will enter in as a factor to cause the continued preservation of a heterozygous condition as a consequence of selection for a certain set of characters.

It remains to consider those portions of the *Nicotiana* literature which deal specifically with the Mendelian inheritance of the characters which we have investigated, and to harmonize our results with those which have been reported previously. Unfortunately there have not been many investigations in *Tabacum* which have been prosecuted far enough to arrive at a definite factor analysis of the differences under consideration. The investigations of Miss Howard (1913), promise of the continuation of which has not thus far been fulfilled, in general confirm those which we have presented in this paper. On the strictly analytic side, however, Miss Howard did not carry her work very far. This doubtless was due to the difficulty of making a factor analysis of the characters which she selected for study, viz., (1) time of flowering, (2) height of stem, (3) arrangement of the leaves on the stem, (4) length of the decurrent portion of the lamina, (5) venation of the leaf, (6) leaf shape, and (7) undulation of the surface and margin of the leaf. For most of these characters she demonstrates, by the presentation of numerical data in some cases as far as F_4, the probability of the character differences in question depending upon multiple factor differences. In the case of height certain of her cultures strongly suggest the existence of a pair of allelomorphs, which has a relatively great effect, for in some of her cultures there are definite discontinuous height differences. For the inheritance of length of the decurrent portion of the lamina Miss Howard postulates the existence of at least three or four distinct pairs of factors. As respects leaf base, she records the synthesis of petiolate types from sessile parents, observing in two cases a simple 1:2:1 segregation into petiolate : intermediate : sessile. As respects corolla color, she records one F_2 population from pink × very pale pink fading into white which consisted of 72 pinks of various shades to 45 whites, but some of the palest pinks were indistinguishable from white. She found evidence of grouping among the pinks, and postulates the existence of two factor differences to account for it. The investigations which we have reported do not throw light upon the factor constitution of the very pale pink varieties with which Miss Howard worked. Our

varieties *angustifolia* and *virginica* have lively pink flowers. Of the paler pinks or "pinkish" forms we have a representative in our *N. Tabacum* var. Cavala, U. C. B. G. 72/05, which has flowers distinctly lighter in color than those of *angustifolia* or *virginica*. Our petiolate forms also seem to be of different constitution from those with which Miss Howard worked, for she presents evidence to show that hers are combinations of recessive factors and that they breed true whenever they occur as segregation products, whereas our petiolate forms often gave plants with sessile leaves as segregation products. We have, however, secured evidence that some distinctly short petiolate forms arise from sessile ones, perhaps by modifications of the AURICULATA leaf type in the direction of stripping the auricle and lower portion of the lamina from such leaves, but our results are not yet definite enough to permit of rigid formulation. Further investigation of the relationships of the various petiolate forms is necessary.

As respects flower color Allard (1919) has presented some interesting data which at first sight appear to contradict those which we have presented. Allard found that carmine × pink gave F_1 carmine and F_2 3 carmine : 1 pink. The back crosses gave consistent data. Thus F_1 carmine × carmine parent gave all carmine, and F_1 carmine × pink parent gave 1 carmine : 1 pink. In F_3 pink segregants bred true for pink, and carmine either bred true for carmine or gave again 3 carmine :1 pink. The difficulty here is that our red is not genetically identical with Allard's carmine. Our flowers of *macrophylla* and *calycina* at full expansion show a color lying between rose red and pomegranate purple of the Ridgway color scale. This color, which we have called red for the sake of brevity, is very close to carmine, but we have another flower color, which we call dark red, represented by *N. Tabacum* var. *macrophylla purpurea,* which is probably identical with the Giant Red flowering tobacco which Allard used in his experiments.

We have made some preliminary tests of this dark red, and find that it behaves differently from red. Crossed with our white it gives dark red in F_1, instead of pink as was obtained from red × white. Since our white carries the factor **R**, which is responsible for the production of pink flower color, dark red must differ from pink in a dominant factor. If we call this factor pair **Pp**, then our various colors of tobacco would have the following genotypes:

Dark red..**WWRRPP**
Red..**WWrrpp**
Pink..**WWRRpp**
White..**wwRRpp**

Obviously this formulation would account for Allard's results without contradicting those which we have presented, but inasmuch as our experimental evidence is not yet complete we refrain from any further discussion of the consequences of this scheme save one. Allard presents certain data for a cross of carmine × white which gave in F_1 light carmine, and in F_2 3 colored : 1 white, the colored being various shades of carmine and pink. Allard's discussion of this case is somewhat mixed, but he evidently erroneously expected a simple monohybrid segregation of the 1 : 2 : 1 kind. That more than one factor is concerned in the cross is clearly shown by the results of crossing some of the extracted whites with pink varieties. The results of three such crosses gave:

1. Pink (Maryland Mammoth) × Extracted white...................36 carmine : 18 pink
2. Extracted white × Pink (Maryland Mammoth)...................20 carmine : 23 pink
3. Pink (Conn. Broadleaf) × Extracted white..........................12 carmine : 39 pink

    Totals..68 carmine : 70 pink

In (1) above we have combined in the carmine class 17 *carmine* and 19 *somewhat lighter than carmine.*

If we consider a cross of dark red × white according to the genetic formulation given above, the F_1 should be dark red, and F_2 should consist of 9 dark red : 3 pink : 4 white. Doubtless the pinks and the dark reds would exhibit various shades, but the three classes should be distinct. If we combine "carmine" and "lighter than carmine" to form a carmine class and dark and light pink to form a pink class, Allard's F_2 data reduce to the following form:

    149 carmine : 64 pink : 65 white.

This ratio compares very favorably with a 9 : 3 : 4 expectation, viz.:

    157 dark red : 52 pink : 69 white.

No F_3 results from sowings from colored F_2 plants are given, but the single F_2 white, which gave when crossed with pink approximately equal numbers of carmine and pink flowering plants, is accountable for as of the genotype **wwRRPp**. Further investigations are in progress for the purpose of determining precisely the relation of dark red and pinkish to the red, light pink, and white colors reported upon in this paper.

There are other references in the literature to Mendelian inheritance in *Tabacum*, but inasmuch as these do not bear upon the characters which we have attempted to analyze it does not appear necessary to discuss them at this point.

VII. SUMMARY

Studies of three intervarietal crosses in *Tabacum* demonstrate that:

1. All the differences between varieties of *Tabacum* can be analyzed in a Mendelian fashion, if sufficient refinement in methods be introduced.

2. Stable recombinations of parental characters can readily be obtained with three or four generations of self-fertilization.

3. Characters outside the range between the parents are sometimes produced following hybridization, and these may be readily established in stable lines by self-fertilization.

4. The petioled leaf base of *angustifolia* and the sessile leaf base of *macrophylla* differ in at least three pairs of factors.

5. A single factor difference exists between normal and split hose-in-hose flowers.

6. Two pairs of factors account for the relation existing between red, light pink, and white flower color. A third pair of factors is necessary to account for dark red.

On the theoretical side it has been pointed out that:

1. Derivation of relationships and erection of systems of classification after the manner of Comes and Anastasia cannot be relied upon unless supported by experimental evidence.

2. An adequate scheme of classification should be based upon identities and dissimilarities in the genotypes, irrespective of the derivation of the forms in question.

3. Mendelian analysis in *Tabacum* requires that special attention be paid to residual portions of the genotype, so that the factor differences under consideration act in a stable residuum most favorable for emphasis of the character differences under investigation.

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EXPLANATION OF PLATES

A special note is due the illustrations in this paper. The line drawings were made by Miss Anna Hamilton and Miss Helen M. Gilkey. Special attention was paid to accuracy in proportions and details. No attempt, however, was made to represent the characteristic *Nicotiana* pubescence. The photographs require no special mention save that sometimes the garden number given in the legend does not correspond with that given on the label in the photograph. The difference is due to a change in system of numbering used in F_3 and in subsequent populations. In this paper, in order to avoid confusion, garden numbers from the beginning have been made to conform to this change. The legends of all the plates have been made more complete than is usual in order to facilitate cross-reference and to enable the reader to grasp their essential significance more readily.

PLATE 55

Fig. 1. *Nicotiana Tabacum* var. *angustifolia*, U. C. B. G. 68/07. A typical plant of *angustifolia* at the height of its blooming period. The laterals overtopping the central axis and the long-petioled *stenophylla* form of leaf are especially to be noted. The drooping of the leaves is very characteristic of this variety.

Fig. 2. *Nicotiana Tabacum* var. *macrophylla*, U. C. B. G. 22/07. A typical plant of *macrophylla* at the height of its blooming period. Note especially the stout laterals overtopping the central axis and the *sessilifolia* type of leaf.

Fig. 1

Fig. 2

Nicotiana Tabacum var. *angustifolia,* U. C. B. G. 68/07. Line drawings of typical details of *angustifolia.* In the upper right-hand corner the characteristic straplike sessile leaf or bract of the inflorescence. Upper left, details of bud, flower, and capsule. Lower right, details of pistil and stamens. Lower left, the typical long-petioled *stenophylla* leaf of *angustifolia.* Leaves $\times$ ½ ; flowers and capsules natural size.

Nicotiana Tabacum var. *macrophylla,* U. C. B. G. 22/07. Line drawings of typical details of a plant of *macrophylla,* showing floral details and the extreme variations in leaf size and shape on the plant. Leaves × ½; flowers and capsules natural size.

Fig. 1. *Nicotiana Tabacum* var. *angustifolia*, U. C. B. G. 68/07. Typical leaves of *angustifolia* of the *stenophylla* type showing the range of variation on a single plant.

Fig. 2. *Nicotiana Tabacum* var. *macrophylla*, U. C. B. G. 22/07. Typical leaves of *macrophylla* of the *sessilifolia* type showing the range of variation on a single plant.

Fig. 1

Fig. 2

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Angustifolia-macrophylla series, F_1 leaves.

Fig. 1. Typical leaf of $10F_1H_2P_{56}$, an F_1 of the *angustifolia-macrophylla* series. Note the short, winged petiole and the clasping auricles.

Fig. 2. Typical leaf of $10F_1H_3P_{58}$, a variation from the usual *latifolia* type of the F_1 leaf. Note the shorter petiole, less conspicuously winged condition, and the almost total lack of auricles.

Fig. 3. Typical leaf of $10F_1H_4P_{55}$. The petiole is somewhat longer than that normal for the F_1.

Fig. 4. Typical leaf of $10F_1H_5P_{56}$. The petiole here is shorter than that normal for the F_1.
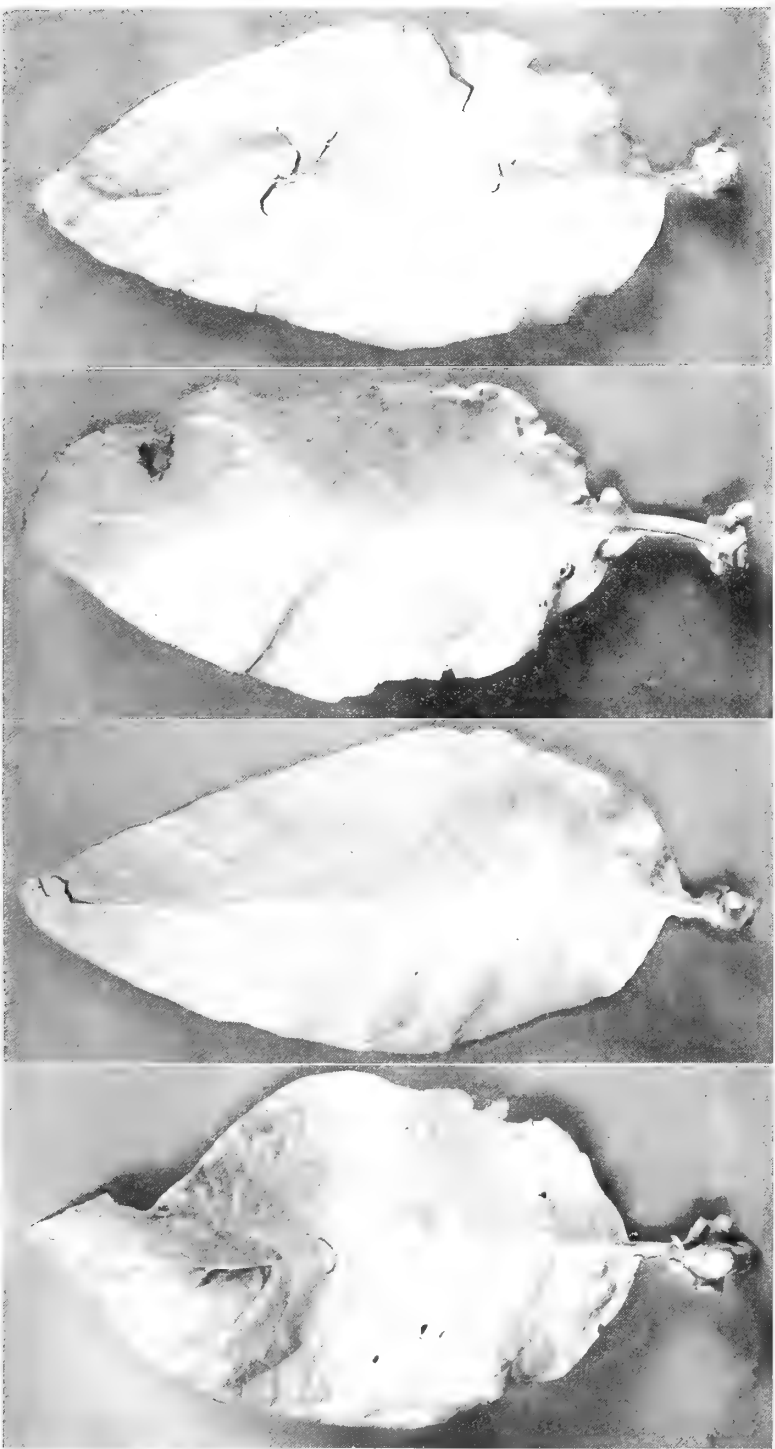
Fig. 1

Fig. 2

Fig. 3

Fig. 4

Portions of inflorescences of *N. Tabacum* var. *macrophylla*, *N. Tabacum* var. *angustifolia* and the F₁ hybrid between them.

Fig. 1. Left, portion of inflorescence of *macrophylla*, middle, of the F₁, and right, of *angustifolia*.

Fig. 2. Left, portion of the inflorescence of *macrophylla*, middle, two of the F₁, and right, of *angustifolia*.

Fig. 1

Fig. 2

PLATE 61

Angustifolia-macrophylla series, F₁ plants.

Fig. 1.   Photograph of $10F_1H_2P_{56}$, the F_1 plant from which the leaf shown in plate 59, figure 1, was taken.

Fig. 2.   Photograph of $10F_1H_5P_{56}$, the F_1 plant from which the leaf shown in plate 59, figure 4, was taken.

[534]

Fig. 1

Fig. 2

Angustifolia-macrophylla series, line drawings of F_1.

Line drawings showing morphological details of the typical F_1 plant of the *angustifolia-macrophylla* series. The garden number of the plant was $10F_1H_{15}P_7$. Leaves $\times$ $\frac{1}{2}$; flowers and capsules natural size.
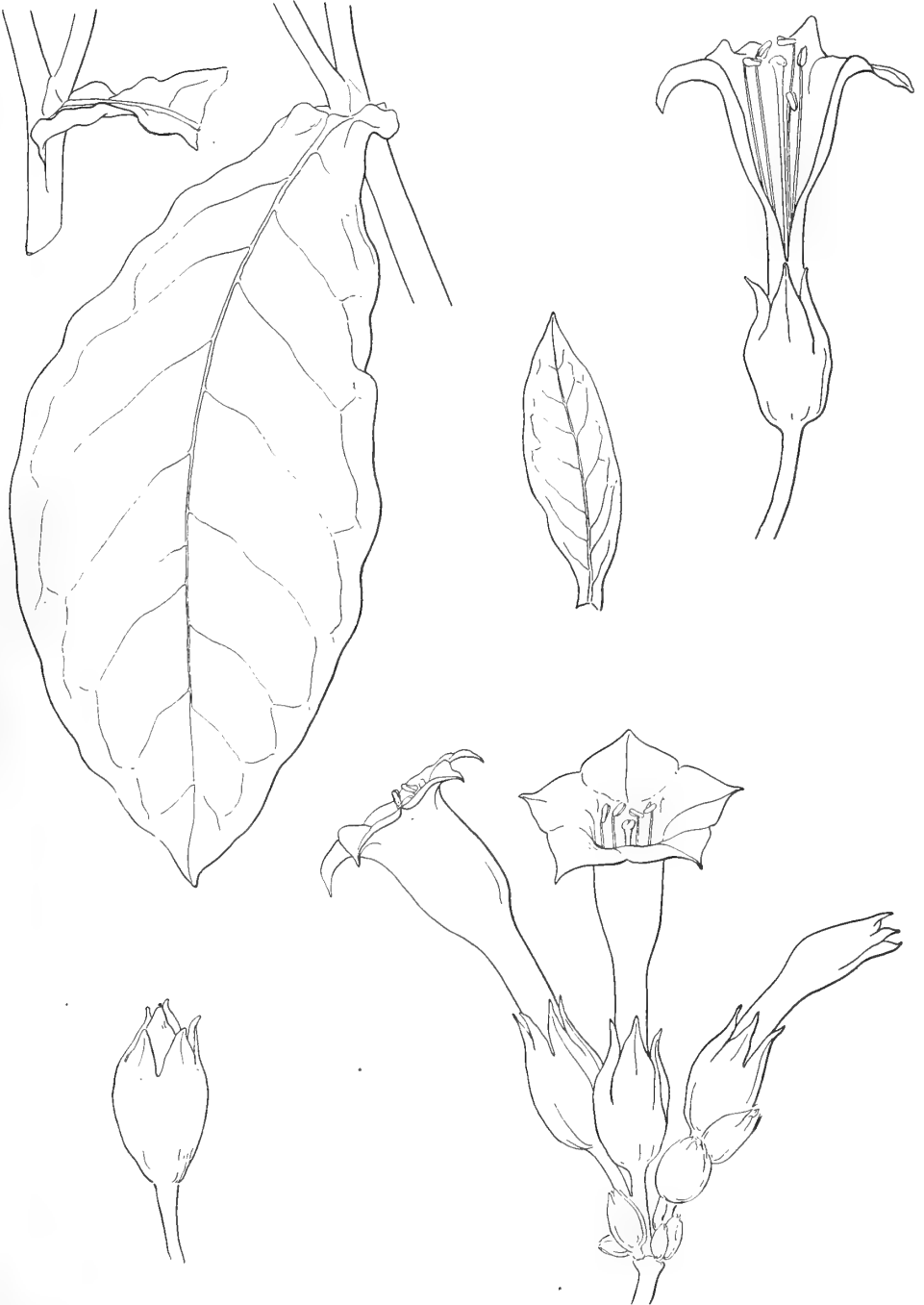
PLATE 63

Angustifolia-macrophylla series, type 1.

Line drawings of morphological details of F_2 of type 1. The garden number was $11F_2H_2P_7P_{49}$. Note particularly the *stenophylla* type of leaf. Leaves $\times$ $\frac{1}{3}$; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 16 *stenophylla* of type 1 and 9 *lanceolata* of type 14.

PLATE 64

Angustifolia-macrophylla series, type 2.

Line drawings of morphological details of F_2 of type 2. The garden number was 11$F_2H_2P_3P_{30}$. The leaf is of the *latifolia* type. Leaves $\times$ $\frac{1}{3}$; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 12 *latifolia*, 8 *sessilifolia*, and 4 *auriculata*.

Angustifolia-macrophylla series, type 3

Line drawings of morphological details of F_2 of type 3. The garden number was $11F_2H_2P_3P_{14}$. The leaf is of the *latifolia* type. Leaves $\times$ $\frac{1}{3}$; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 4 *latifolia* and 10 *sessilifolia*.

PLATE 66

Angustifolia-macrophylla series, type 4.

Line drawings of the morphological details of type 4. The garden number was $11F_2H_2P_7P_{18}$. The leaf is an extreme form of the *latifolia* type. Leaves $\times$ $\frac{1}{3}$; flowers and capsules natural size.

No progeny was grown from this plant.

PLATE 67

Angustifolia-macrophylla series, type 5.

Line drawings of morphological details of type 5. The garden number was $11F_2H_4P_{41}P_{14}$. The leaf is of the *latifolia* type. Leaves $\times \frac{1}{3}$; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 24 *latifolia* and 1 *auriculata*.

PLATE 68

Angustifolia-macrophylla series, type 6.

Line drawings of morphological details of F_2 of type 6. The garden number was $11F_2H_4P_2P_{18}$. The leaf is of the *latifolia* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 5 *stenophylla*, 17 *latifolia*, and 4 *auriculata* plants, but the segregation was not distinct.

Angustifolia-macrophylla series, type 7.

Line drawings of morphological details of F_2 of type 7. The garden number was $11F_2H_2P_{1J}P_{4N}$. The leaf is of the *latifolia* type. Leaves $\times$ ⅓; flowers and capsules natural size.

The F_3 progeny of this plant was uniformly of the same type as the parent, and the line bred true in subsequent generations.
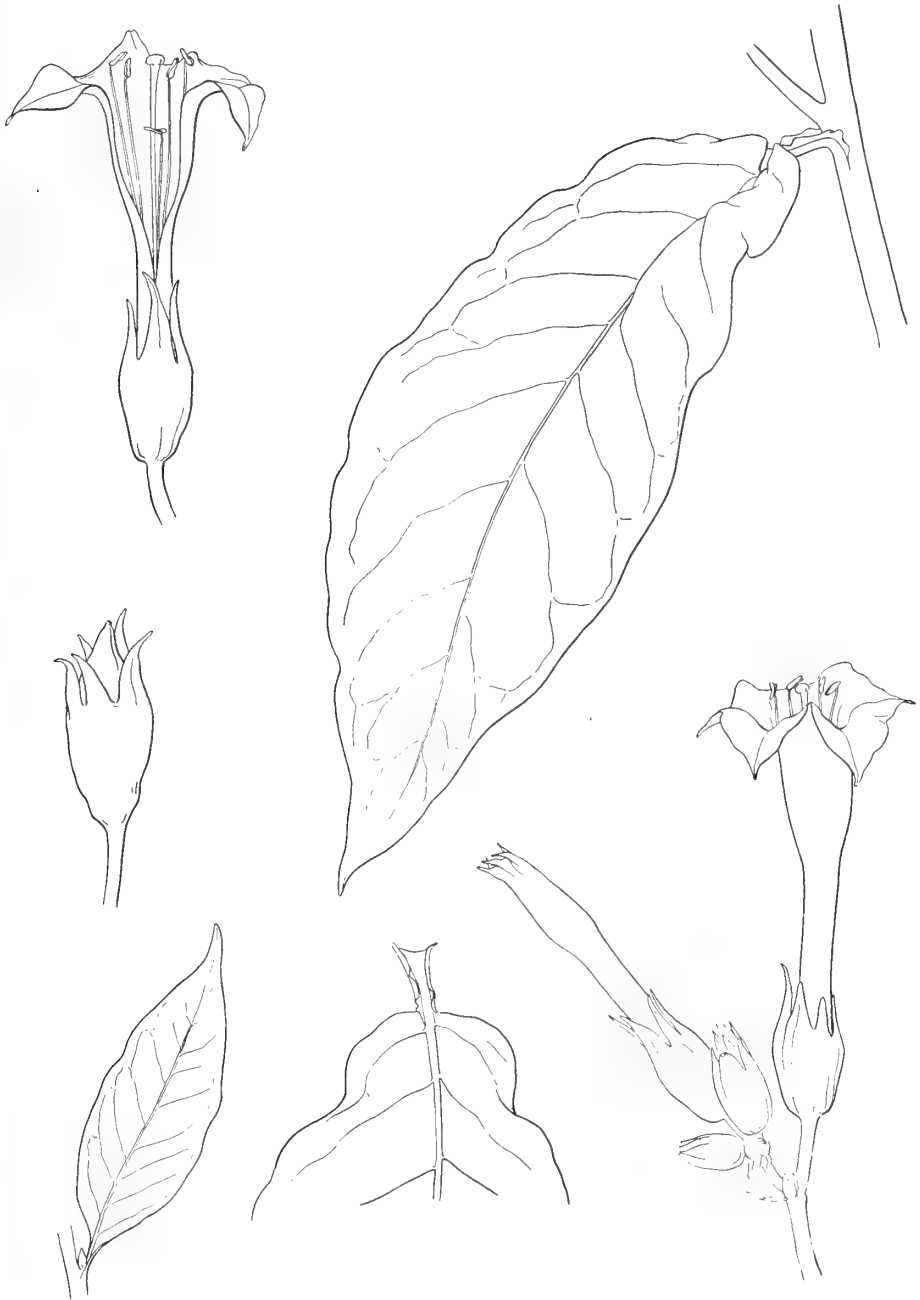
PLATE 70

Angustifolia-macrophylla series, type 8.

Line drawings of morphological details of F_2 of type 8. The garden number was $11F_2H_2P_3P_{41}$. The leaf approached the *auriculata* type. Leaves $\times$ $\frac{1}{3}$; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 16 *sessilifolia* and 8 *auriculata*, indicating that the F_2 plant was an extreme variant of the heterozygous *sessilifolia-auriculata* condition.
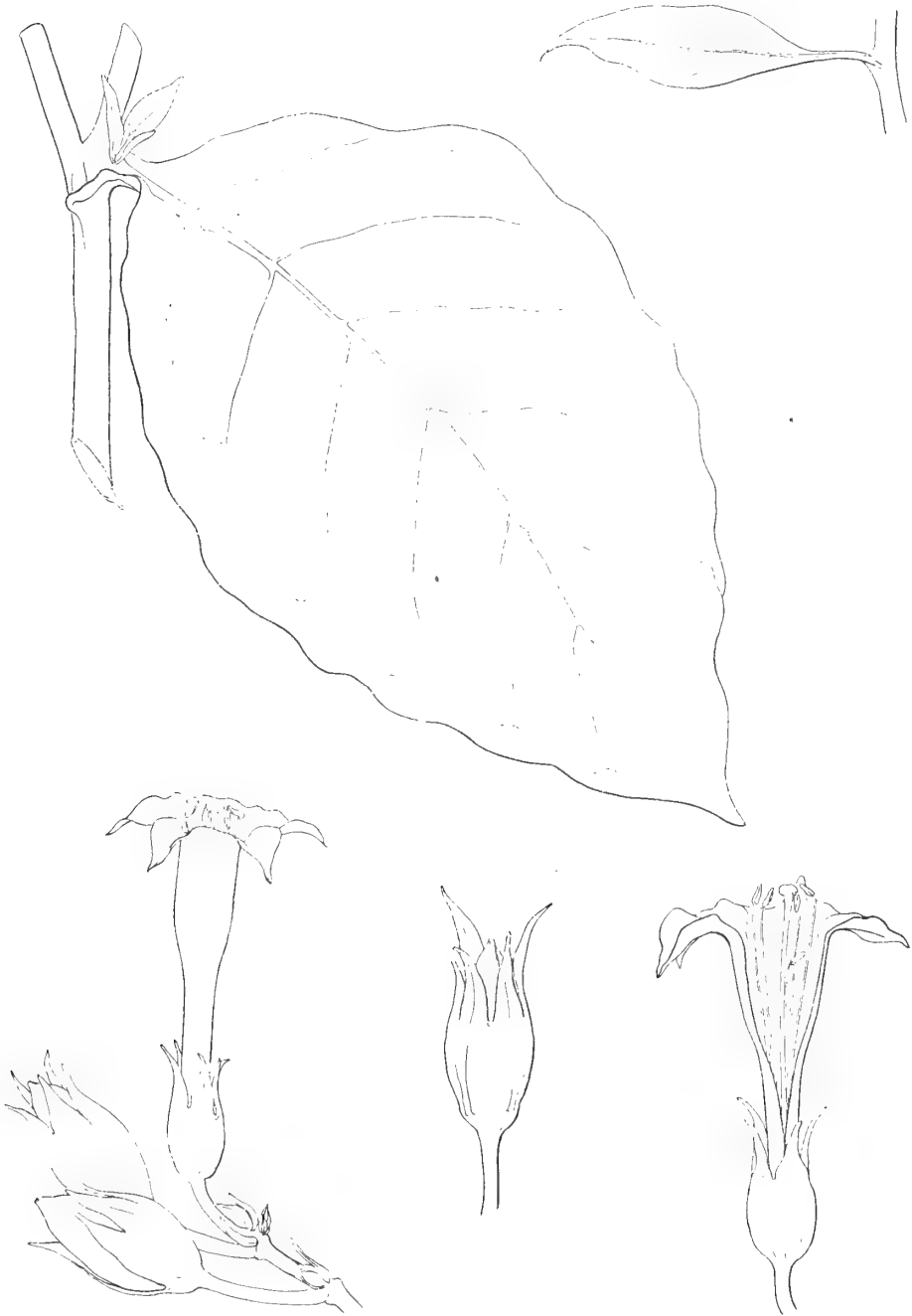
PLATE 71

Angustifolia-macrophylla series, type 9.

Line drawings of morphological details of F_2 of type 9. The garden number was $11F_2H_4P_{41}P_8$. The leaf is of the *latifolia* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 18 *latifolia* and 7 *sessilifolia*.
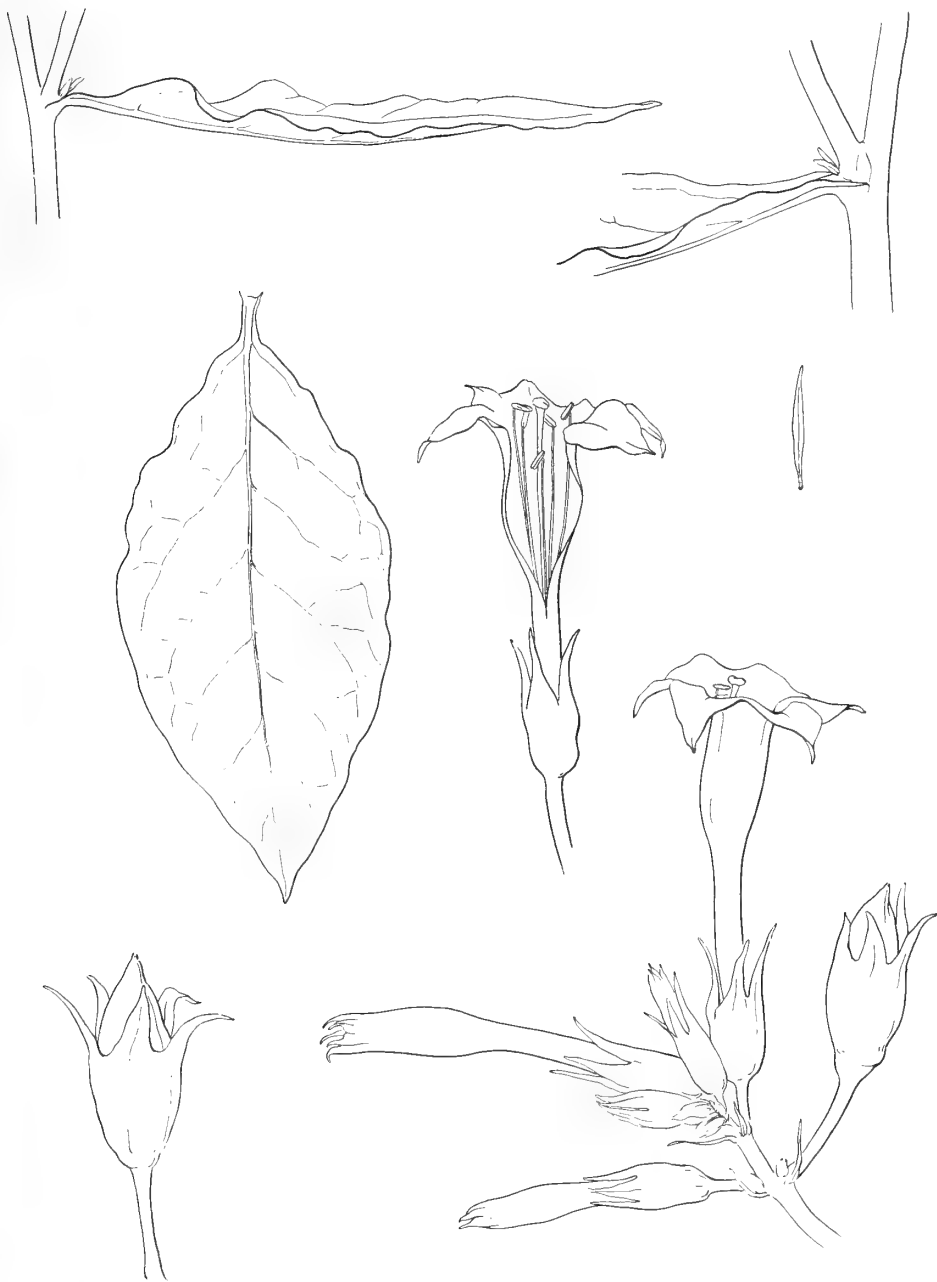
PLATE 72

Angustifolia-macrophylla series, type 10.

Line drawings of morphological details of F_2 of type 10. The garden number was $11F_2H_4P_{41}P_{17}$. The leaf was of the *auriculata* type. Leaves $\times$ ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted uniformly of *auriculata* plants.
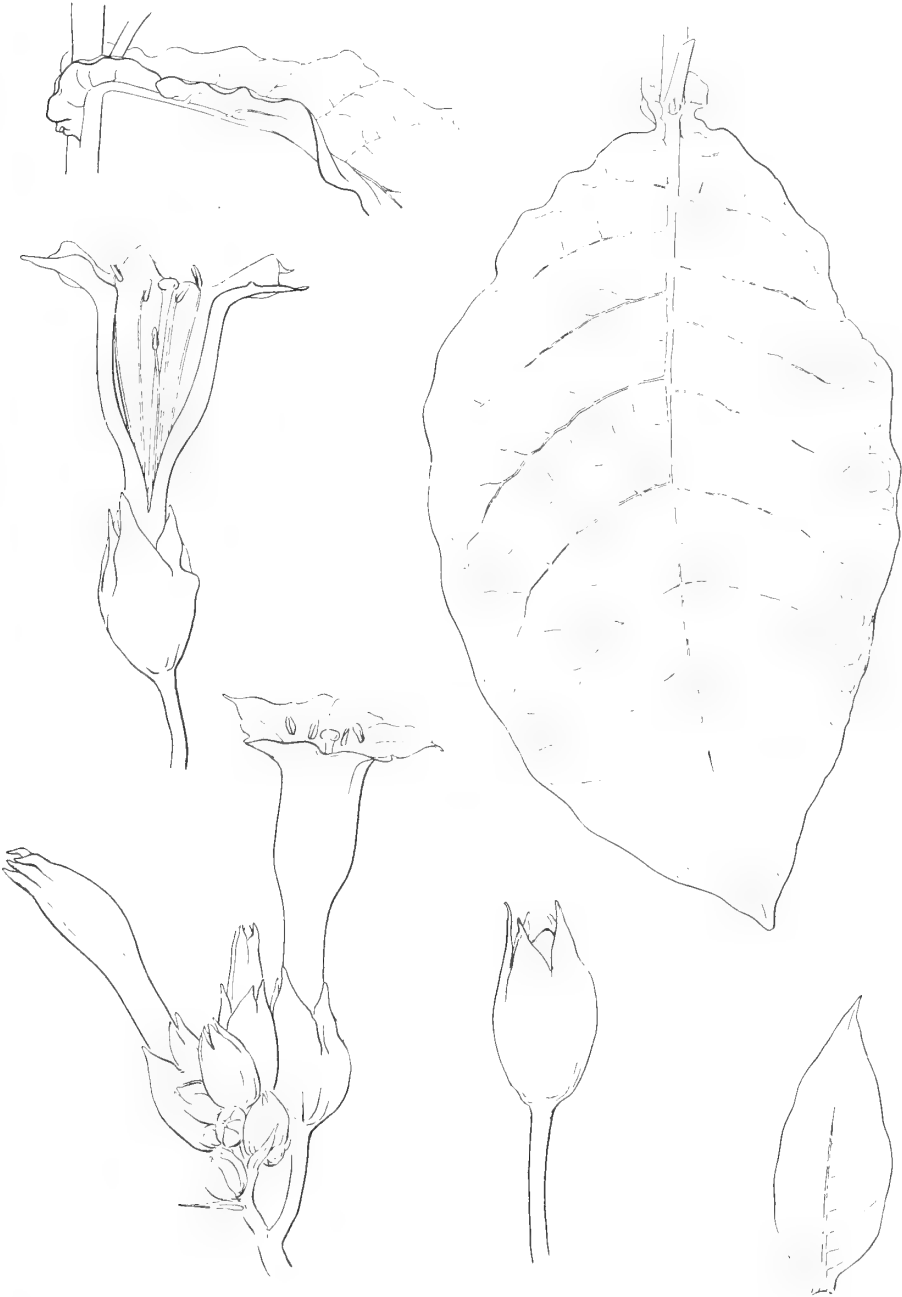
PLATE 73

Angustifolia-macrophylla series, type 11.

Line drawings of morphological details of F_2 of type 11. The garden number was $11F_2H_4P_{41}P_9$. The leaf is of the *sessilifolia* type. Leaves $\times$ $\frac{1}{3}$; flowers and capsules natural size.

The F_3 progeny of this plant was uniformly of the same *sessilifolia* type.
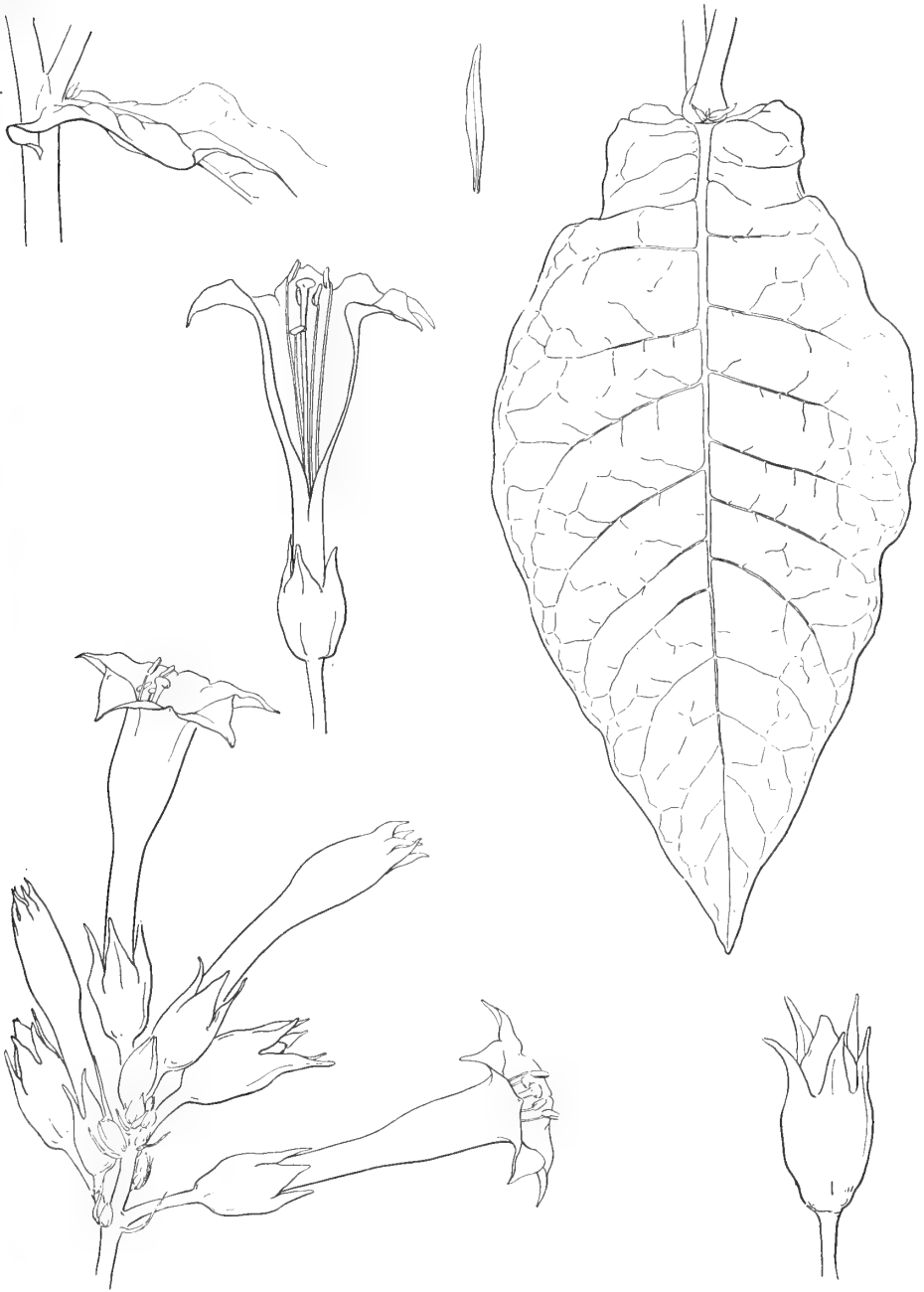
PLATE 74

Angustifolia-macrophylla series, type 12.

Line drawings of morphological details of F_2 of type 12. The garden number was $11F_2H_4P_{41}P_{12}$. The leaf is of the *loriifolia* type. Leaves $\times$ ⅓; flowers and capsules natural size.

The F_3 progeny of this plant was uniformly of the same *loriifolia* type, and two constant races, one with red and one with light pink flowers, were obtained from it.

[560]

PLATE 75

Angustifolia-macrophylla series, type 13.

Line drawings of morphological details of F_2 of type 13. The garden number was $11F_2H_2P_3P_{44}$. The leaf is of the *lanceolata* type. Leaves $\times$ $\frac{1}{3}$; flowers and capsules natural size.

The F_3 progeny of this plant was uniformly of the same *lanceolata* type.

PLATE 76

Angustifolia-macrophylla series, type 14.

Line drawings of morphological details of F_2 of type 14. The garden number was $11F_2H_2P_3P_{3s}$. The leaf was classified as *sessilifolia*, although strictly it is intermediate between *sessilifolia* and *lanceolata*. Leaves $\times$ $\frac{1}{3}$; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 24 plants of the same *sessilifolia* type and 1 "filler," which had leaves more like *auriculata* of type 8.

PLATE 77

Angustifolia-macrophylla series, type 15.

Line drawings of morphological details of F_2 of type 15. The garden number was $11F_2H_2P_3P_{10}$. The leaf is of the *sessilifolia* type. Leaves $\times$ ⅓; flowers and capsules natural size.

The F_3 progeny of this plant was uniformly of the same leaf type.

Line drawings of morphological details of F_2 of type 16. The garden number was $11F_2H_2P_3P_{80}$. The leaf is of the *sessilifolia* type. Leaves $\times$ ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 15 *sessilifolia* and 8 *auriculata*.

PLATE 79

Nicotiana Tabacum var. *calycina,* U. C. B. G. 110/05.

Line drawings of morphological details of leaf and flower of *calycina.* The leaf is of the *lanceolata* type, and the flowers are of the conspicuously teratological, split hose-in-hose form. Leaves × ½; flowers and capsules natural size.

Nicotiana Tabacum var. *virginica*, U. C. B. G. 78/05.

Line drawings of morphological details of leaf and flower of *virginica*. Contrast the normal flowers and auricled leaves with the corresponding details of *calycina*, shown in plate 79. Leaves × ¼; flowers and capsules natural size.

Nicotiana Tabacum var. *alba*, U. C. B. G. 30/06.

Line drawings of morphological details of flower and leaf of *alba*. Note especially the rugose leaf. Compare this drawing with those of *macrophylla* shown in plates 57 and 58, figure 2. Leaves $\times$ ⅓; flowers and capsule natural size.
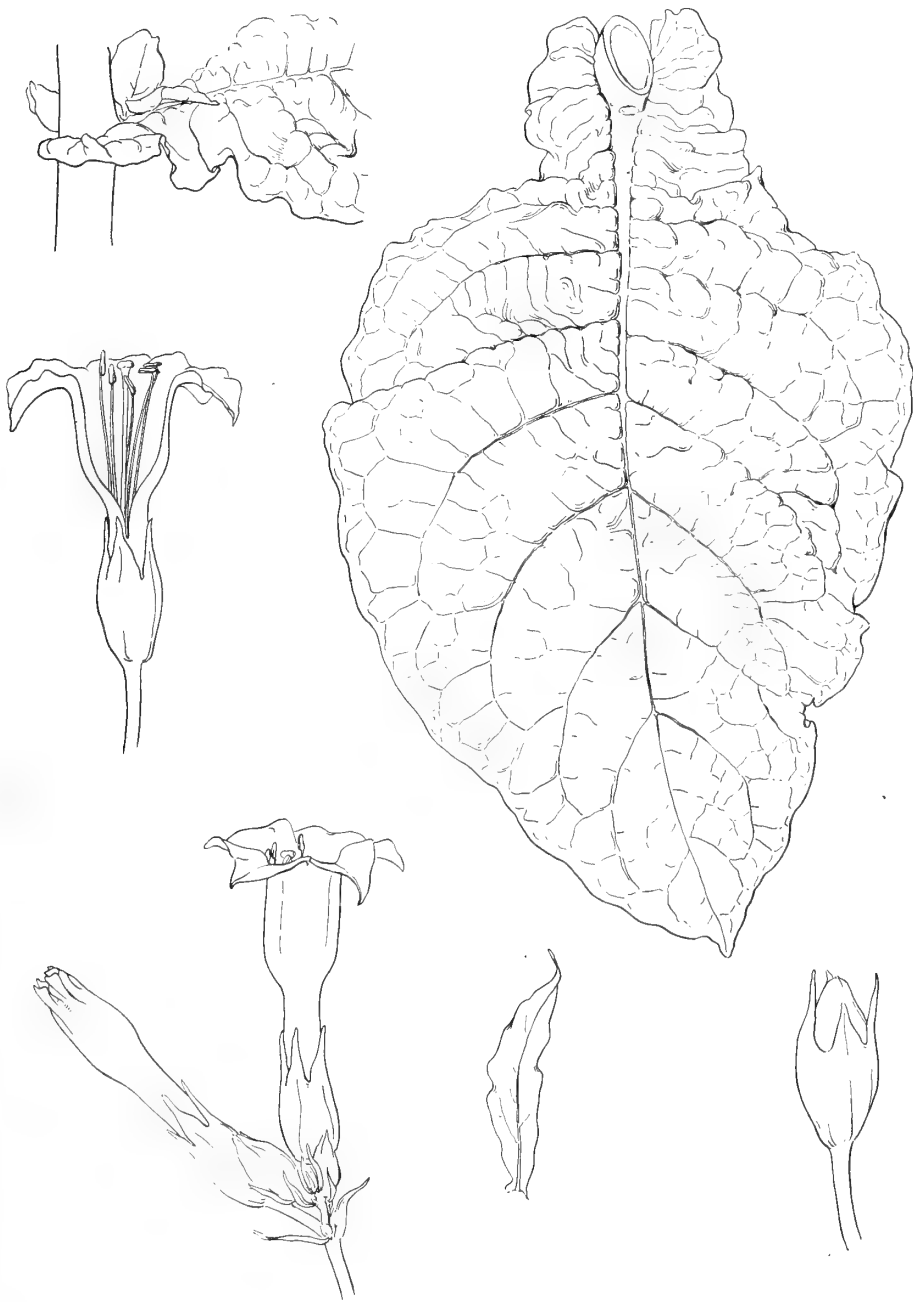
PLATE 83

Fig. 1. *Calycina-virginica* series, an F_1 plant. A typical F_1 plant of the *calycina-virginica* series. This plant is at the height of its blooming period. The garden number was $10F_1H_1P_{51}$.

Fig. 2. *Alba-macrophylla* series, an F_1 plant. Photograph of a typical F_1 plant of the *alba-macrophylla* series. The garden number was $10F_1H_{24}P_{54}$. The plant is at the height of its blooming period.

Fig. 1

Fig. 2

PLATE 84

Alba-macrophylla series, F₁ leaves.

Fig. 1. Photograph of typical leaves of *alba*.

Fig. 2. Photograph of a typical leaf of $10F_1H_{25}P_{17}$, F₁ of the *alba-marcophylla* series.

Fig. 3. Photograph of a typical leaf of $10F_1H_{24}P_{34}$, F₁ of the *alba-macrophylla* series.

Compare these leaves with those of *macrophylla*, shown in plate 58, figure 2. The rugoseness of *alba* has been carried over, to a somewhat reduced extent, into the F₁ hybrid.

Fig. 1
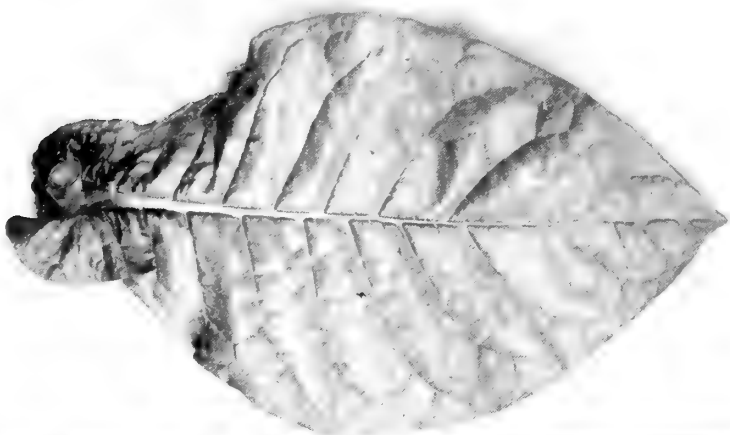
Fig. 2
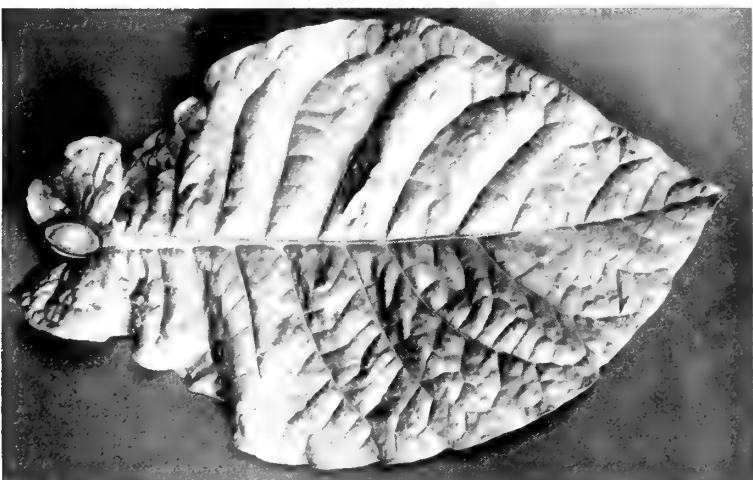
Fig. 3

PLATE 85

Fig. 1. *Alba-macrophylla* series, F_2 plants. Photograph of two adjacent plants, $11F_2H_{24}P_{34}P_{22}$ and $11F_2H_{24}P_{31}P_{23}$, from the same F_2 population of the *alba-macrophylla* series. An illustration of segregation for height in this population.

Fig. 2. *Alba-macrophylla* series, an F_4 plant. Photograph of a typical F_4 plant, $13F_4H_{24}P_{34}P_{23}P_2P_{50}$, of a dwarf line of the *alba-macrophylla* series. The line here illustrated was derived from the dwarf F_2 plant shown in figure 1. This line has bred true for seven generations.

Fig. 1

Fig. 2